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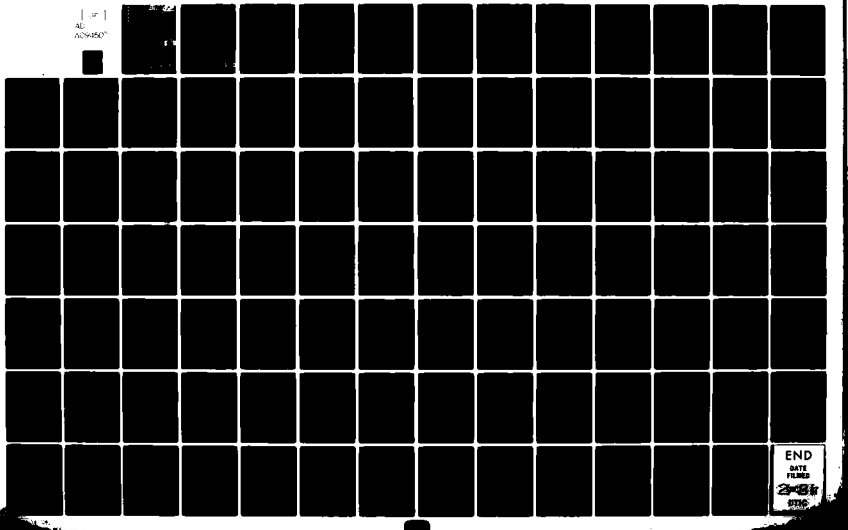

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BIOLOGICAL EFFECTS OF SHORT, HIGH-LEVEL EXPOSURE TO GASES: NITROGEN OXIDES

PHASE REPORT

PREPARED BY

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July 1980

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U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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Nitrogen oxides	Concentration	Intermittent	Toxicity
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Nitric oxide	Acute	Effects	Biological
Exposure	Chronic	Behavioral	Health
			Criteria

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report presents an analysis of the available literature describing health and performance effects of exposure to nitrogen oxides (NO_x). The US Army's concern is with high-level, short-term exposures that may exceed present threshold limit values of the American Conference of Governmental Industrial Hygienists: .5 ppm (9 mg/m^3) as a TWA; intended changes are a TWA of 3 ppm (5.4 mg/m^3) with a short term exposure limit of 5 ppm (9 mg/m^3) for 15 minutes.

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Dose-response relationships were developed for intensities of exposure from the highest (fatal) level to thresholds of minimal response. It is concluded that NO_x can in general be equated to nitrogen dioxide (NO_2) because this is much more toxic than the only other oxide of importance, nitric oxide (NO), which oxidizes to NO_2 in air. If NO is present in equal or greater concentration, an allowance for its effect is recommended. NO_2 exhibits mainly two sets of toxicological characteristics: immediate irritancy and delayed, cumulative tissue damage. Proven effects are mainly on the respiratory tract.

Intense exposures result in death, hospitalization with recovery, or severe responses not requiring hospitalization. Survivors may be free from permanent effects. This range covers concentrations from 20 ppm (38 mg/m^3) to 200 ppm (376 mg/m^3) and up, for single exposures of a few minutes up to 1 hour.

At the other extreme, the threshold of measurable respiratory impairment in volunteers is below 2 ppm (3.8 mg/m^3) in sensitive subjects. Spontaneous recovery from single exposures is probably complete. Intermittent exposures are apparently similar in effect to continuous exposure at the same level and for the same total time, and may result in irreversible lung damage. The influence of reversible or irreversible respiratory impairment by NO_x on oxygen sufficiency and, hence, physical and mental capacity has not been measured in man, but effects such as reduced maximal work time are possible. An effect thoroughly studied in animals is enhanced mortality from experimental respiratory infection in conjunction with NO_x exposure; it is not known if this is predictive of increased susceptibility to infection in humans.

The effects of exposures causing significant discomfort are the least well documented. Lacrimation, coughing, and respiratory distress may be expected in a minute or so at about 50 to 100 ppm (94 to 188 mg/m^3).

The most conspicuous areas of uncertainty are:

- Quantitative significance of impaired ventilation and oxygen transport in terms of physical and mental performance
- Applicability to human response of the enhanced susceptibility to respiratory infection observed in animals
- The effects of intermittent exposure on development of long-lasting or irreversible lung damage

There is insufficient evidence for quantitative estimates of the consequences of exposure below the level of severe discomfort. Until there are further studies, it would be prudent to minimize exposure to NO_x.

EXECUTIVE SUMMARY

The overall purpose of this project is to characterize the biological responses to short, high-level exposures to four gases associated with certain Army weapons systems (ammonia, carbon monoxide, sulfur dioxide, and the nitrogen oxides). This report analyzes and synthesizes the available literature concerned with possible health and performance effects of exposure to nitrogen oxides. Military personnel may experience exposures that are brief, relatively intense, perhaps repeated, and not covered by existing standards for occupational protection over a working lifetime. The conditions specified for this study of nitrogen oxides were an exposure intensity outside existing standards, duration of 1 hour or less, and repetition up to 6 times per day and up to 14 days.

The scope of work was to review published information, analyze it for biological effects at various levels of exposure, and seek dose-response relationships to assist formulation of guidelines. Attention was to be given to the extrapolation to man of data from animal experiment or human exposure in different conditions, the consequences of repeated vs. single exposure, and the interaction of nitrogen oxides with other toxicants and stresses. The report was to present conclusions including suggested health criteria and to identify major deficiencies in current information.

NO_2 is a hazardous toxicant characterized by two kinds of biological response:[2] (1) immediate irritation of the respiratory tract and other mucosa and (2) delayed tissue damage, principally in the deep lung (alveoli and bronchioles). NO is considerably less toxic. Other nitrogen oxides are not of toxic significance in the military setting.

Accidental exposure of humans to harassing or dangerous concentrations of nitrogen oxides has occurred in activities such as the burning or detonation of explosives in confined spaces; welding, brazing, and oxyacetylene cutting; industrial plant operations involving nitration, acid dipping, etc.; firefighting; and silo filling.

Data from animal experiments, a few reasonably reliable estimates of human exposure in accidents, and volunteer exposures all form a consistent picture. Typical effects of NO_2 exposure are: at very high level, death (early or delayed); at high level, hospitalization with reversible or irreversible lung injury; at moderate level, sensory irritation interfering with performance; at moderate or low level, respiratory impairment (reversible or irreversible), possibly impairing performance, and possible increase in susceptibility to infectious agents and respiratory allergens.

Response in animals at the level of severe to fatal effects can be expressed in the form $Ct^n = K$, where K is a constant for a given level of response in a given species, C is the concentration of NO_2 (ppm), t is the time of exposure (minute), and n is calculated to be less than one (< 1.0) for the rat. The same dose-response relationship holds for the enhancement, by low-level exposure to NO_2, of respiratory infection in mice.

The threshold of strong sensory discomfort, sufficient to hinder normal activities, is believed to be about 1 minute at 50 ppm or 5 minute at 25 ppm. Reversible respiratory impairment is measurable in volunteers to below 5 ppm and 10 minute exposure, but it is not known if there is a significant effect on physical endurance or mental ability at this level. Animals show increased susceptibility to respiratory infection after a single exposure at 3.5 ppm for less than 1 hour or several exposures at 1.5 ppm, but it is not known if this occurs in man.

The evidence about intermittent exposure is conflicting, but the balance tends to indicate that, in the specified range of conditions, intermittent exposures have a similar effect to continuous exposure at the same concentration and for the same total duration of exposure. It is prudent not to expect extensive recovery in the intervals between exposure as defined for this project, except that acute respiratory impairment will subside.

Important information gaps concern: (1) the effects of respiratory impairment by NO_2 on physical endurance and mental ability; (2)whether the potentiation of respiratory infection, observed in animals, occurs in man; (3) consequences of intermittent exposure; and (4) cumulative effects of low-level exposure.

The evidence from long-term animal exposures at low concentrations is highly suggestive of human risk of cumulative and irreversible lung damage. Coupled with the suspected but unproven risk that brief exposure may cause physical/mental impairment (reversible, possibly also irreversible) and enhanced susceptibility to respiratory infection, the evidence suggests that the prudent course is to avoid prolonged or repeated exposure to nitrogen oxides.

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I. INTRODUCTION AND BACKGROUND

Military personnel may experience occupational exposures to toxic gases beyond the range of existing standards and guidelines. Exposures are likely to be brief, relatively intense, and repeated; for this project they were defined as not more than 1 hour, repeated up to 6 times per day and for 1 to 14 days. The available occupational standards deal with exposure of workers for a working lifetime of 40-hour weeks. Such limits are set at a "safe" margin below the threshold of demonstrated or suspected minimal harm (including mere discomfort). The standards do not deal with the quantitative consequences of exceeding time-weighted average concentrations or ceiling concentrations by given increments of concentration or time. There are also various short-term or emergency exposure limits that present guidelines for somewhat more intense exposure, perhaps including unpleasant but nonserious responses; these usually apply only to single events. These standards and guidelines do not touch on exposure conditions typical of military occupational hazards, such as the brief and repeated relatively intense exposures to propellant fumes that may occur in weapon firing from a turret.

For the military problem, one needs to know the probable consequences of higher level exposures, through successive zones of increasing response that may be described, for example, as threshold response, mild response, severe response, and immediate danger. This information can be used in designing equipment for protection of its users, in testing performance of equipment, and in making decisions involving emergency exposures.

The federal standard for occupational exposure to NO_2 is 5 ppm* for an 8-hour time-weighted average. The Occupational Safety and Health Administration (OSHA), in setting this standard (29 CFR 1910.1000, FR 39:23542, 27 June 1974), based it on a recommendation by the American Conference of Governmental Industrial Hygienists[4] (ACGIH) but omitted the ACGIH's designation of 5 ppm as a ceiling limit rather than an average concentration. The National Institute for Occupational Safety and Health[69] (NIOSH) recommended reducing the occupational standard to 1 ppm NO_2 ceiling (15-minute sample).

The American Industrial Hygiene Association[5] (AIHA) recommended occupational emergency limits for NO_2; these limits are such that single exposures can be tolerated without adverse health effects but not

*Throughout the remainder of the report, NO_2 concentrations are expressed as parts per million (ppm). To convert ppm to mg/m^3, multiply by 1.8.

necessarily without acute discomfort. The recommended limits were 35 ppm NO_2 for 5 minute, 25 ppm for 15 minute, 20 ppm for 30 minute, and 10 ppm for 60 minute.

The Environmental Protection Agency's standard for NO_x is 0.053 ppm annual average (FR 36(84) Part II: 8186-8201, 1971). In 1977, the World Health Organization[98] recommended a short-term public exposure limit of 0.1-0.17 ppm for 1 hour, not to occur more than once a month. The National Academy of Sciences[67] had earlier (1971) recommended a short-term public limit of 1 ppm NO_2 and a public emergency limit (occasioning some temporary discomfort but no injury) of 5 ppm for 10 minute, 3 ppm for 30 minute, and 2 ppm for 60 minute.

The ACGIH[4] recommended a threshold limit value of 25 ppm for NO and OSHA adopted this as a federal standard of 25 ppm NO for an 8-hour time-weighted average (29 CFR 1910.1000, FR 39:23592, 27 June 1974). NIOSH[69] recommended keeping this standard and noted that the only other occupational standard was the German Democratic Republic's maximum allowable concentration of 16 ppm NO.

The health effects of only two nitrogen oxides, nitric oxide (NO) and nitrogen dioxide (NO_2) were investigated. There is no data concerning the presence or absence in military vehicles of nitrous oxide (N_2O), or the trioxide and pentoxide (N_2O_3 and N_2O_5) although the latter is important in air pollution as a photochemical intermediate.[68,69,98] NO in air oxidizes to NO_2, but the rate depends on the square of the NO concentration and is quite slow at low concentrations except in photochemical reaction involving ozone. Since NO is readily formed in high-temperature combustion, it is an abundant component at the source of many emissions, but its reaction with oxygen means that it is never encountered without NO_2 and it may be largely oxidized to NO_2 before being inhaled. NO_2 is also considerably more toxic. A study of the health effects of nitrogen oxides, NO_x, therefore becomes mainly a study of NO_2 with lesser attention to NO.

NO_2 is soluble in water and is absorbed in the upper respiratory tract where it dissolves in water to form dilute nitric acid (HNO_3), which is an irritant to the mucous membranes. It also penetrates to the deep lung where it can produce pathologic changes. Its properties lead to two distinct categories of response: (1) immediate irritation, which may be attributed to the formation of strong (highly ionized) acid, and (2) oxidative destruction of cellular components and connective tissue. Toxicologically, it behaves like other gases that combine early irritancy with delayed tissue destruction, e.g. chlorine (Cl_2), phosgene ($COCl_2$), and ozone (O_3).

Accurate information about gas concentrations in exposures to NO_x is difficult to get for two reasons: (1) exposure is often to a mixture of nitrogen oxides and perhaps other compounds, and (2) analytical methods may be deficient in accuracy or in distinguishing between compounds.

NO_x mixtures are formed in experimental generation by methods such as the reaction of nitric acid with copper, in which concentrated acid gives mainly NO_2 and dilute acid gives mainly NO, in both cases accompanied by nitric acid vapor. In accidental exposures associated with very high temperatures (oxyacetylene flame, electric arc), NO is predominant. Fossil fuel combustion and explosives form NO and NO_2 in similar amounts.

Errors in sampling and analysis include variable collection efficiency, variable efficiency of conversion to a colored compound for measurement, conversion of NO_2 to NO in sampling, and failure to analyze NO_2 and NO separately. Better methods and techniques began to be introduced about 20 to 25 years ago and reliable observations are now attainable with proper care; work more than 25 years old is generally questionable.

The Griess-Ilosvay reaction, which forms a colored azo compound, is the basis of many methods, of which Saltzman's[79] is generally preferred for NO_2 analysis. The gas-air mixture may be sampled directly into the reagent solution or collected in an absorbent and reacted later. Sampling efficiency depends on design of apparatus, flow rate, etc., all of which must be carefully standardized. Conversion of NO_2 to azo dye is not complete, so that a factor must be used in calculating concentrations; this too will vary if conditions are not standardized. Other wet chemical methods that are acceptable include the sodium arsenite method (subject, however, to interference by NO) and the TGS-ANSA method (not interfered with by common air contaminants). The National Institute for Occupational Safety and Health[69] recommends a method in which NO_2 is absorbed in triethanolamine on a solid substrate for subsequent reaction with Saltzman reagent, NO passing unabsorbed is oxidized by CrO_3 to NO_2, and this NO_2 is then collected in triethanolamine or directly in Saltzman reagent. Efficiency for NO_2 and NO is good at lower concentrations but falls off, e.g., it is 97.4 percent for NO at 8.6 ppm and 67 percent at 50 ppm. Calibration is necessary for each set of different experimental conditions. The Saltzman method has been adapted for continuous monitoring in many variations, but the instrumentation is troublesome in operation and error prone.

Instrumental methods based on the chemiluminescent reaction of NO with O_3 have come into favor since 1970 and are generally accepted. Frequent calibration and carefully controlled techniques are essential. For NO_2 analysis, the gas is catalytically reduced to NO. Other compounds may interfere, but this source of error can be controlled in laboratory experiments. Other instrumental methods are based on gas

chromatography and electrochemistry. The approach preferred by many investigators today is to use a chemiluminescent monitor for continuous recording of concentrations and to check it by manual Saltzman-method samples. For chemical analysis of mixed NO_2 and NO, one approach is to collect NO_2 first, then oxidize NO and collect the NO_2 (as in the NIOSH method described above). The alternative is to take two samples, analyze one for NO_2 and the other for total NO_x after oxidizing NO; the NO is then calculated by difference. This is satisfactory if the NO is more than a small proportion of the total NO_x.

II. APPROACH TO THE PROBLEM

The approach to the work involved the following major steps:

Identification of information sources

Preliminary screening of information before acquisition

Assessment of the availability of sufficient literature to perform remaining work elements

Acquisition of the literature

Critical review of documents for scientific validity

Evaluation of biologic response data in terms of behavioral, performance, and health effects, both immediate and delayed, and reversible and irreversible

Identification of gaps in information and development of suggestions for follow-on work.

The main sources of information were the various computer data bases, especially MEDLINE and its back files, TOXLINE, TOXBACK, NTIS, and NIOSHTIC. The computer search was performed by first selecting key terms describing substance, exposure, and response:

Nitrogen oxides	Dose	Effects	Biological
Nitrogen dioxide	Acute	Behaviorial	Health
Nitric oxide	Chronic	Performance	Criteria
Exposure	Intermittent	Toxicity	
Concentration	Continuous	Physiology	

All materials yielded by the search of the data bases were screened to identify articles apparently relevant to the study. The screening was based on the content of article abstracts (when available) and on the presence of keywords in article titles. Full-text copies of all apparently relevant articles were then secured for critical review and evaluation. In addition to confirming the relevance of the material to

the present study, the critical review involved determinations of the adequacy and appropriateness of the experimental design, the accuracy and validity of the statistical analyses performed, and the correctness of the conclusions in light of the data analysis.

Most of the source material used in the project was recent and therefore in the data bases. Earlier work was sought through the references in recent reviews of nitrogen oxides and by tree searching from individual articles. Work from more than 20 to 25 years ago was not generally sought except for any indications of quantitative response in humans, for which the total body of evidence was scanty except at the lowest concentration levels. Some early papers on animal experiments were reviewed because they have been widely quoted, even in recent reviews, but they were almost totally rejected as unreliable.

Analysis of the information focused initially on the types of biological effects that had been observed in humans or were likely on the basis of animal experiments. The next step was to seek quantitative data that would support development of dose-response models. Such data were found in animal response at lethal concentrations and at the other extreme of low-level responses, and a single model was found to fit both sets.

Finally, the information gaps that had been identified were summarized and suggestions were developed for their closing and for reinforcing areas of weakness.

The report is divided into the following sections:

- "Summary of Effects and Conclusions," which presents the main findings of the literature review in summary fashion and identifies significant gaps and inconsistencies.
- "Discussion" of the data presented in the literature, leading to the main findings, gaps, and inconsistencies.
- A section on "Suggested Follow-on Work," in which possible additional research is proposed to fill in major gaps in the information or to resolve discrepancies.
- "Literature Review and Analysis" (Appendix A), which is a presentation of the purpose, methods, and findings of each key article, followed by a critical analysis, as appropriate, of the experimental design, statistical methods, and the correctness of the conclusions.

III. SUMMARY OF EFFECTS AND CONCLUSIONS

A. EXPOSURE TO NO_x

The type of exposure to NO_x envisaged by the scope of work was from combustion of explosive or propellant nitro compounds in enclosed spaces. The duration and frequency were specified to be not more than 1 hour on each occasion, repeated up to 6 times per day for 1 to 14 days. No data on the likely range of exposure intensity were found in a search assisted by U.S. Army scientists and librarians, but the possibility of exposure is generally acknowledged. The toxicologic data indicate that response to NO_x effectively includes only NO_2 and NO, and that the latter may be neglected unless its concentration is greater than that of NO_2. Unless otherwise stated, the following summary refers to NO_2.

B. BIOLOGICAL EFFECTS

The emphasis in this section is on qualitative evidence of the biological effects that have been observed in humans or are considered likely from observations in animals. The next section emphasizes observations and published conclusions on quantitative aspects.

Proven or likely effects of NO_2 exposure are overwhelmingly in the respiratory system. They result from two toxic mechanisms: irritation from formation of nitric and nitrous acids, and damage from reaction with lung cells and connective tissue. The irritant reaction is immediate and reversible; tissue damage is characteristically delayed and may be slowly reversible or irreversible. Effects on other organs and systems such as histopathologic and hematologic changes are indicated by some evidence but they are probably of minor signficance within the scope of this report and, since they are not quantifiable, would not assist in the development of exposure criteria.

Effects in the lung vary in nature and intensity with the intensity of exposure, and they may be immediate or delayed. An immediate effect is defined here as one that usually occurs at once or within an hour of a single exposure. Delayed effects are of two kinds: those occurring more than two days after a single exposure and those not occurring until two days or more after the onset of continuous exposure or intermittent exposure.

The immediate effect of the most intense exposures is early death from respiratory spasm or pulmonary edema.[64] Exposure at high sublethal concentrations induces pulmonary edema requiring hospitalization,[58,64] and there is some evidence, mainly from animal experiments, that a single brief exposure of this intensity may cause persistent lung damage such as emphysema and interstitial fibrosis.[40,50] Severe discomfort, perhaps with temporary incapacitation from lacrimation, coughing, and respiratory distress, is induced by exposures somewhat less intense than to require hospitalization, and there may be nonclinical pulmonary edema with

reversible respiratory impairment.[46,63,64] Single exposures of intensity causing slight or tolerable discomfort in humans have been shown in animal experiments to cause reversible enhancement of susceptibility to respiratory infection and to aggravate allergenic reactions.[23,34,61] Low concentrations, at or below the threshold of sensory perception and below the current federal occupational standard (5 ppm), may cause reversible impairment of respiratory functions, but it is not known whether this is accompanied by a significant effect on physical or mental performance.[1,94] The most sensitive indicator of exposure to NO_2 may be impairment of dark adaptation, but the published data require confirmation.[9,80]

The effects of an intense exposure, with or without hospitalization, may subside and be followed by delayed and serious effects as much as two or three weeks later, including death or incapacitation from infectious pneumonia or obstructive pathologic changes in the lung.[58,62,64] There may be persistent lung damage in survivors. In less intense exposure, the obstructive changes and accompanying impairment of respiratory functions may undergo complete remission without clinical intervention.[17,43] Continuous or intermittent exposure at moderate or low concentrations induces in animals cumulative lung damage, reversible or irreversible, and increasing susceptibility to respiratory infection when artificially induced.[8,24,32] The likelihood of similar responses to human exposure is not known.

C. CONCENTRATION AND DURATION OF EXPOSURE

The development of exposure criteria requires data on quantitative response to various combinations of concentration and duration of exposure. One source of such data is mortality in groups of animals subjected to lethal exposures.[12,21,40,50] These consistently show that concentration is more important than duration in determining the level of mortality; for example, if the concentration is doubled, the time for equal effect is less than one-half. Data suitable for statistical analysis show that, for single exposures of duration from a few minutes to one day, the relationship $Ct^n = K$ holds good. In this expression, C is concentration (usually in ppm), t is duration (minutes), n is less than unity and is constant for a given species, and K is constant for a given level of effect (e.g., 50 percent mortality) in that species. There are no firm data from accidents causing human death or incapacitation that could be used to test the applicability of the model. However, a few estimates of exposure intensity permit rough comparisons that suggest that the human is not exceptionally sensitive or resistant to dangerous exposures compared with other animals.[3,46,70]

Animal exposures do not lend themselves to numerical estimates of immediate irritant response in humans, but there are a few data on volunteer exposures.[56,63] The American Industrial Hygiene Association[5] used the lethality model to estimate a range of occupational emergency exposure limits (EELs) from human responses at two single points (50 ppm NO_2 for 1 minute, 25 ppm for 5 minutes).[63] The

recommended EELs were 35 ppm for 5 minutes, 25 ppm for 15 minutes, 20 ppm for 30 minutes, and 10 ppm for 60 minutes.

Volunteer exposures at lower intensities, causing no more than slight discomfort if any, have been designed and reported in such a way that they cannot be used to test the concentration-time relationship.[1,93,93] However, animal experiments on enhanced mortality from respiratory infection after NO_2 exposure have yielded abundant data that have been shown to fit the same model.[55] Calculations in the present report suggest that the exponent of time, n, is similar for this effect, which can be observed in mice after 30 minutes exposure to 3.5 ppm NO_2,[34] and for lethality in several species, which requires about 100 to 200 ppm for the same duration.[12,40,50]

There are several gaps in information that limit the development of exposure criteria. One is the very scanty data on human response at levels of sensory discomfort or incapacitation in acute exposure to NO_2. The evidence is limited to one laboratory study with acceptable concentration estimates, accidents with questionable exposure data, and early work with unreliable chemical analyses. Another uncertain area is the practical consequences of respiratory impairment. It is not known if the impairment of respiratory functions that has been observed in volunteers at low levels of exposure implies any significant degradation of military performance. The evaluation of intermittent exposure is also uncertain. It appears that, in some circumstances, intermittent exposure over a given period is similar in its effects to continuous exposure over the same period and at the same concentration;[23,24,33] this is seen in histopathologic lung changes and in enhancement of respiratory infection. However, there is insufficient information to support a comprehensive set of criteria.

Several other observations have a bearing on the quantitative interpretation of exposures to nitrogen oxides. Volunteer exposure has shown that the acute irritant effects of NO_2 are independently additive to those of SO_2.[1] Exposure of animals to CO, sufficient to give carboxyhemoglobin concentration of about 25 percent, did not significantly affect mortality from NO_2 exposure.[50] Concurrent exposure to NO_2 and inert respirable particles enhanced the toxic effect[66] and this resembles observations with other toxicants. Physical stresses such as pre-exposure chilling or postexposure exercise may enhance the toxicity of NO_2.[50] Variations in species sensitivity have a bearing on the applicability of animal experiments to human response. Within one set of experiments, the exposure for 50 percent mortality in rats was twice that for guinea pigs; mice and dogs were intermediate.[50] However, experiments with the same species by different investigators have shown larger variation.[12,21]

The quantitative significance of exposure to NO is uncertain because it oxidizes in air to NO_2. Even though the reaction rate is low at low concentrations, it appears to be effectively impossible to inhale a pure NO-air mixture. NO is certainly much less irritant and tissue damaging than NO_2 and its main toxic effect may be in reaction with hemoglobin to reduce its oxygen-carrying capacity.[43,68] The National Institute for

Occupational Safety and Health recommended an exposure standard of 25 ppm NO.[69] In comparison with the federal standard of 5 ppm NO_2, this reflects an estimated fivefold difference in toxicity, which suggests that NO would not be of practical importance unless present in greater concentration than NO_2.

IV. DISCUSSION

A. SINGLE BRIEF EXPOSURE TO NO_2

The discussion of single exposure deals first with the types of biological effects that have been observed in humans and then with those in animals. In each case, the treatment is selective, focusing on effects that may be of significance to the soldier in training or combat and especially on effects that are usable in the development of quantitative dose-response relationships later in the discussion.

1. Biological Effects in Humans

A selection of data on human response to exposure to NO_2 is presented in Table 1.

a. Guidelines for Emergency Exposure

The American Industrial Hygiene Association[5] recommended occupational emergency limits of 35 ppm NO_2 for 5 minutes, 25 ppm for 15 minutes, 20 ppm for 30 minutes, and 10 ppm for 60 minutes. The definition of an emergency exposure limit was that it can be tolerated without adversely affecting health but not necessarily without acute discomfort or other evidence of irritation or intoxication; impairment of vision, judgment, and coordination may occur, but not so as to prevent self-rescue. The AIHA stated that the limits are for use by specialists in industrial hygiene; they should not be used as indexes of comparative toxicity or as fine lines dividing dangerous from tolerable levels of contamination and must not be extrapolated to other durations, especially below 5 minutes.

In 1971, the National Academy of Sciences[67] (NAS) recommended short-term public limits, intended to protect the most susceptible segment of the population, of 1 ppm NO_2 for 10, 30, or 60 minutes exposure and 0.5 ppm for a 5-hour day on 3 or 4 days per month. The NAS also recommended public emergency limits, which envision the possibility of some temporary discomfort but no injury, of 5 ppm NO_2 for 10 minutes, 3 ppm for 30 minutes, and 2 ppm for 60 minutes.

b. Lethal and Incapacitating Exposures

Nitrogen dioxide is an occupational hazard encountered in a wide variety of activities and has caused many fatalities and serious incapacitations. The activities involved have included welding, brazing, oxyacetylene cutting, and other operations with high-temperature flames;[3,60,70] burning or detonation of explosives in confined spaces;[54] fires and firefighting;[42] industrial operations such as nitration and acid dipping;[62] and silo filling.[58]

TABLE 1. Human Exposure to NO_2

Conc., ppm	Time, min	Effects	Reference
250	4.7	Collagen degradation, methemoglobinemia, tightness in chest, dyspnea, nonproductive cough, retrosternal burning sensation	Hatton et al.[46] (1977)
158	10	Intolerable; coughing, irritation of nasal and laryngeal mucosa; lacrimation; headache; nausea, vomiting. Subsided after 7 hours, no delayed or long-term effect	Lehmann et al.[56] (1913)
70	20	2/2 hospitalized, pulmonary edema; 1 retired on disability	Mangold et al.[60] (1971)
60	60	Laryngeal irritation; increased respiration rate	Lehmann et al.[56] (1913)
25-100	120	Marked mucosal irritation; increased pulse and respiratory rate	Lehmann et al.[56] (1913)
45 (with 90 ppm NO)	30 followed by 15	Subject hospitalized 7 days, pulmonary edema; no clinical sign at 73 days	Norwood et al.[70] (1966)
20 (with 80 ppm NO)			
50	1	Pulmonary discomfort; nasal irritation (more intense than at 25 ppm for 5 min); substernal pain (2/7 subjects)	Meyers et al.[63] (1961)
25	5	Pulmonary discomfort	Meyers et al.[63] (1961)
5	15	Threshold for decreased arterial partial pressure of O_2 and decreased diffusion capacity for CO in bronchitics	von Nieding et al.[93] (1973)
4-5	10	Lung compliance decreased and airway resistance increased in healthy subjects	Abel[1] (1967)
1-6	15	Threshold for increased airway resistance in bronchitics	von Nieding et al.[93] (1973

Typical consequences of fatal or incapacitating exposure, as described in a clinical review by Milne,[64] are: immediate death, with sudden collapse and cyanosis, from respiratory spasm and anoxia; early death, during or within hours of exposure, from pulmonary edema; delayed death or incapacitation, from delayed edema (several days) or from bronchiolitis obliterans or infective pneumonia (few weeks); and reversible incapacitation, from respiratory distress typical of irritant gas exposure, with no persistent overt symptoms but possible long-term lung damage.

A recently reported high-level exposure is the accident in which three Apollo-Soyuz astronauts were exposed for approximately 4.7 minutes to an estimated average NO_2 concentration of 250 ppm (Hatton et al.[46]). Symptoms one hour later included tightness of the chest, retrosternal burning sensation, inability to inhale deeply, and nonproductive cough. Discomfort was worse the next day and the subjects were unable to participate in forced expiratory volume tests. The subjects were asymptomatic on the third day. The reported responses are somewhat less intense than might be expected from other observations and estimates of human and animal responses, and so the intensity of exposure may be questioned. It was stated, for example, that the space vehicle's atmosphere cleared very rapidly; perhaps the effective exposure time was less than 4.7 minutes or the crew were exposed to less than the calculated average concentration because of incomplete mixing of the cabin atmosphere before ventilation.

Many other reports confirm the general picture of acute primary irritation and development of pulmonary edema, followed in some cases by delayed development of lung damage. Adley[3] reported an accidental exposure for 23 minutes to NO_x in the range from 200 to 400 ppm; one of the four men died 10 days later from pneumonia. The composition of the gas is uncertain because it was oxidized to NO_2 for analysis and it must have contained NO, having been formed in a high-temperature flame. Furthermore, chemical analysis at that time (1946) was not reliable. However, the report is of interest because Adley exposed himself for shorter periods (up to 7 minutes) in reconstructions of the accident; he experienced only coughing and chest tightness. Norwood et al.[70] reported a similar accident, which was reconstructed with sampling, that suggested that the worker's exposure averaged 45 ppm NO_2 and 90 ppm NO over a 30-minute period with a subsequent 20 ppm NO_2 and 80 ppm NO for 15 minutes. About 18 hours later, the worker's forced expiratory volume was normal, but his vital capacity was 50 percent of normal. He was hospitalized for 7 days with diagnosed pulmonary edema and recovered uneventfully.

Lowry and Schuman[58] first described "silo-filler's disease" and identified NO_2 as its cause. They described it as characterized by acute irritation, early pulmonary edema, failure to recover completely, and delayed bronchiolitis fibrosa obliterans (BFO) after 2 to 3 weeks. The delayed onset of BFO typically develops rapidly and with little warning. For example, McAdams[62] reports a case in which a person who

was exposed to NO_x from red fuming nitric acid did not consult a physician until the 23rd day and was dead of BFO on the 27th day.

Exposures of sublethal intensity result in a range of symptoms, mainly of respiratory irritation. The most detailed report is that of Hasegawa (Lehmann and Hasegawa[56]), who exposed himself to the limit of tolerance and experienced coughing, irritation of the nasal and laryngeal mucosa, lacrimation, headache, nausea, and vomiting. The symptoms subsided after 7 hours and he reported absence of delayed or long-term effects. At lower levels of exposure, Hasegawa experienced mucosal irritation and increased pulse and respiratory rates. Unfortunately, the NO_2 must have been mixed with significant amounts of NO and nitric acid (it was generated from nitric acid and copper) and the method of analysis was unsuitable for distinguishing between these and NO_2; the analysis is also questionable in its estimate of total NO_x concentration. The NO_2 concentrations generally quoted for this work (Table 1) are probably overestimates and are used in this report only for lack of better data on humans.

Meyers and Hine[63] gave a brief report on volunteers exposed to 50 ppm and 25 ppm NO_2. Within 1 minute at 50 ppm, 2 out of 7 subjects left the chamber complaining of severe substernal pain. About half of the subjects found exposure to 25 ppm NO_2 for 5 minutes to be unpleasant but tolerable.

c. Low-Level Exposures

Low-level exposures are those causing little or no discomfort although they may be immediately perceptible. Most of the reported observations are at 10 ppm NO_2 or below for less than 1 hour; some reports include observations above this level.

Von Nieding has investigated the threshold of physiological response to NO_2 exposure (also NO) in a series of studies. His subjects have been volunteers with chronic nonspecific lung disease, also referred to as "bronchitics," and healthy volunteers, and they have included a range of ages, both sexes, smokers, and nonsmokers. Laboratory-grade NO_2 of high purity has been used, and exposure has been monitored by Saltzman's method.[79] The experiments have focused on concentrations of 5 ppm NO_2 or less and durations of 15 minutes, but other conditions have been used. Physiological observations have included airway resistance, arterial partial pressure of oxygen, and CO diffusing capacity. Changes in these and other respiratory functions appear to be fully reversible after single exposures to NO_2.

Similar results were reported by Abe[1] who exposed five healthy subjects for 10 minutes to NO_2 at 4 to 5 ppm. The laboratory-grade NO_2 was monitored by Saltzman's method.[79] Pulmonary functions were measured before exposure, immediately after exposure, and at 10, 20, and 30 minutes after exposure: these functions were effective compliance (i.e., the distensibility of the lung), inspiratory resistance, and expiratory resistance. Compliance decreased and resistance increased, but

little change was seen until after exposure. Significant changes occurred at 0 to 10 minutes after exposure and continued to increase until observations were discontinued after 30 minutes postexposure. The extent of the changes showed significant impairment of pulmonary function. Spirometer readings of forced expiratory volume before and 30 minutes after exposure showed no change. The lack of effect may be explained by the observation that forced expiratory volume is an insensitive measure of airway constriction.[59] Others have reported increased airway resistance after brief exposures to low concentrations of NO_2, e.g. 6 to 40 ppm for 5 minutes;[66] 0.7 to 2 ppm for 10 minutes;[85] 1.5 to 3 ppm for 45 minutes;[78] 7 to 17 ppm for 10 minutes.[100-102]

An effect of possible significance to military efficiency is impairment of dark adaptation (ability to see on going from bright to dim light). Shalamberidze[80] found dark adaptation impaired at a threshold concentration of 0.075 ppm NO_2, but Bondareva[9] had earlier found none at 0.16 ppm and a positive response at 0.26 ppm. The discrepancy between these reports may be due to uncertainty about the composition of Bondareva's NO_2 and the difficulty of getting reproducible results in this kind of experiment. What the two studies do suggest is the need for further work since the response could impair military performance at exposure levels likely to be encountered. Shalamberidze found NO_2 and SO_2 additive for impairment of dark adaptation.

2. Biological Effects in Animals

a. Lethal and High Sublethal Exposures

The most extensive set of data on response at lethal and high sublethal exposure levels is that of Hine et al.[50] who reported results on several hundred animals of five species. Concentrations ranged from 40 to 250 ppm NO_2 and exposure times from 5 to 1440 minutes. At or above 40 ppm NO_2, eye irritation and respiratory distress were seen. Nearly all deaths occurred within 24 hours and were characterized by bronchiolitis, desquamated bronchial epithelium, infiltration by polymorphonuclear cells, and edema fluid in the alveoli. A few animals died later from pneumonitis and secondary bacterial infection. Interstitial fibrosis occurred in about one-third of the survivors at 30 days and persisted in some up to 6 months.

Carson et al.[12] exposed rats, rabbits, and dogs to 28-416 ppm NO_2 for 5 to 60 minutes. Rats exposed at lethal levels showed severe respiratory distress and eye irritation, and deaths occurred within 30 minutes to 3 days; pulmonary edema was diagnosed. Similar but less marked responses were seen in animals exposed at 50 percent or 25 percent of concentrations causing 50 percent mortality in the same exposure time (LC_{50}) and none died. At 15 percent of LC_{50} values, no pathologic change was seen. Lung/body weight ratios increased in proportion to intensity of exposure at 50 percent and 25 percent but not at 15 percent of lethal exposure levels. The LC_{50} values for rats correspond to those for 75 to 100 percent mortality in the study of Hine et al.[50]

DiPasquale and Davis[21] exposed rats and mice for 5 minutes to 260 to 3280 ppm NO_2. Severe respiratory distress was seen during exposure, and all deaths occurred within 2 days. Gross pathology indicated that death resulted from pulmonary edema with a few animals showing pulmonary hemorrhage.

The pulmonary focus of response to NO_2 has been confirmed in many studies, e.g., by Gray[40] who determined LC_{50} values of 88 to 1445 ppm NO_2 for rats exposed for 2 to 240 minutes and found pulmonary edema, emphysema, and pneumonitis at postmortem but no pathologic signs in other organs. His results for 15 minutes exposure give an LC_{50} for the rat of 420 ppm, which is higher than the 150 ppm of Hine et al. and 201 ppm of Carson et al., possibly because Gray used early (1941, 1943) analytical methods and also because of differences in animal strain and age.

The rat data of Hine et al. were the most suitable to support dose-response estimates at lethal and high sublethal dosage levels. The report gives numbers of animals exposed and dying in each group and includes 335 rats in 32 groups of which 15 had fractional mortality. Data of Hine et al. for mouse, guinea pig, and dog did not give significantly different values of LC_{50} from the rest over the range of 5 to 1440 minutes exposure, but the numbers of animals were smaller; rabbit data were too scattered for satisfactory analysis. Carson et al., DiPasquale and Davis, and Gray reported calculated LC_{50} values without details of numbers dying in each group exposed.

b. Exposures at Lower Concentrations

(1) Pulmonary Effects

Animal experiments show pulmonary function changes similar to those observed in humans. For example, Dowell et al.[22] exposed beagles to 3 to 16 ppm NO_2 for 1 hour. At 7 ppm and up, acute pulmonary edema was observed. Arterial partial pressure of O_2 was depressed and partial pressure of CO_2 raised. Impaired lung compliance was associated with impaired surfactant activity. Guidotti and Liebow[44] exposed dogs to 37 ppm NO_2 for 4 hours by a technique that exposed the left lung and kept the right lung as control. A fall of O_2 uptake to 65 percent in 30 minutes was attributed to regional changes in pulmonary perfusion and airway resistance. The authors observed that the study of NO_2 "is assembling a picture of generalized diffuse alveolar damage."

There have been several investigations of impairment of lung clearance mechanisms. Ciliastasis or impaired mucociliary activity was observed by Cralley[19] in vitro and by Giordano and Morrow[35] in vivo. Stephens et al.[84] and Evans et al.[28] observed loss of cilia in the rat lung after 3 days exposure to 17 ppm NO_2. Macrophage inhibition was observed by Acton and Myrvik[2] and by Hadley et al.[45]

(2) Biochemical and Other Effects

Buckley and Balchum[10] found biochemical changes, principally in enzyme activity of the liver, spleen, kidney, and serum, after acute exposure of guinea pigs to NO_2. Histopathologic changes have occasionally been reported in organs other than the lung, e.g., in the liver, spleen, and heart of squirrel monkeys exposed to 35 to 50 ppm NO_2 for 2 hours (Henry et al.[47]). Ehrman et al.[27] found decreased hemoglobin and erythrocytes, and increased bilirubin and methemoglobin, in mice after 1 hour exposure to 10 ppm NO_2. The investigators interpreted these changes as a hemolytic anemia caused by nitrate and nitrite ions from reaction of NO_2 with water. This suggests the possibility of impairment of tissue oxygenation beyond that caused by decreased pulmonary function.

An effect that has not been confirmed in man is increased susceptibility to allergic sensitization and aggravated response to allergens in sensitized animals, demonstrated by Matsumura[61] in guinea pigs exposed for 30 minutes to 20 to 80 ppm NO_2, exposures that are in the sublethal level.

Wagner et al.[95] found an indication of accelerated tumor formation in a strain of mice susceptible to spontaneous tumors but did not claim a definitive finding. The observation has not been confirmed and is generally discounted, e.g., by Tabershaw and Cooper.[87]

(3) Enhancement of Respiratory Infection

A biologic effect that has been extensively studied in animals is enhancement of respiratory infection by exposure to NO_2. In a typical experiment, mice are exposed to NO_2 and then to a respiratory pathogen. The bacterial challenge is adjusted to give a low level of mortality in control mice, challenged at the same time, that have been exposed to clean air only. Response to NO_2 exposure is recorded in terms of excess mortality in NO_2-exposed mice vs. control mice. The infectivity model is valued as a sensitive and quantifiable tool for assessing response to low levels of exposure such as are encountered in polluted air.

The mechanism of enhancement is relevant to the present study because of its possible bearing on effects in man. Reference has been made above to impairment of lung clearance. This was investigated by Ehrlich[23] who measured the viable count of bacteria in mouse or hamster lungs at intervals after bacterial challenge. The decrease to 30 to 40 percent of initial count in 5 to 6 hours that was observed in control animals was diminished or completely suppressed in animals previously exposed for 2 hours to 5 to 50 ppm NO_2. Goldstein et al.[36] used radioactive bacteria and found that bactericidal activity, measured by viable count, was depressed in conditions that did not significantly depress lung clearance measured by radioactive count. This observation, which has been confirmed by others, is generally accepted to be attributable to impaired macrophage activity. Ehrlich and Henry[24] suggested two other

possible mechanisms for enhancement of respiratory infection: alteration by NO_2 of tissue to provide nutritional factors, and direct stimulation of bacterial growth by NO_2. They considered these factors less likely mechanisms than impairment of phagocytic activity and mucociliary clearance.

Enhancement of mortality from respiratory infection can be demonstrated in mice after exposure to 0.5 ppm NO_2 for about 1 week or to 28 ppm NO_2 for as brief a time as 6 minutes, but there is no clear evidence of a corresponding effect in man. It must be remembered, however, that the enhancement is in animals exposed to a dosage of respiratory pathogen causing fractional mortality in animals not exposed to NO_2. In contrast, Gray et al.[39] found a protective effect of NO_2 exposure in rats subject to spontaneous pneumonia but not experimentally infected.

In most experiments on enhanced respiratory infection, bacterial challenge is made soon after the end of NO_2 exposure, within about 1 hour. If it is delayed, the enhancement may subside. Ehrlich[23] showed that mice exposed for 2 hours to 5 to 25 ppm NO_2 and challenged with K. pneumoniae had a mortality enhancement that was less at 6 hours and absent at 27 hours. In contrast, mice exposed to K. pneumoniae and then to NO_2 showed no significant difference in mortality enhancement when exposure to NO_2 was made at 1, 6, or 24 hours later. It is to be noted that the decay of enhancement in the first group (NO_2 before bacteria) was after brief NO_2 exposure (2 hours); no similar experiments appear to have been made to find how quickly the enhancement subsides after either long NO_2 exposure or several intermittent exposures that might induce a longer-lasting enhancement of mortality.

B. REPEATED BRIEF EXPOSURE TO NO_2

The effects of single brief exposures to NO_2 appear generally to be reversible except at high dosage levels where, for example, Hine et al.[50] found slowly resolving fibrosis in rats surviving exposures. Human exposure at high dosage levels has not been reported to cause irreversible effects in survivors and there is some evidence to the contrary. For example, Gregory et al.[42] followed up 87 percent of the survivors 30 years after the Cleveland Clinic fire in which there were 97 fatalities within 2 hours and 26 more within 30 days, and found no significant difference in mortality from that of unexposed controls. The fire involved large quantities of X-ray film and the toxic atmosphere would have included NO_2, NO, CO, and HCN. However, the negative result from mortality data does not exclude irreversible respiratory damage and increased morbidity.

The question is whether repeated brief exposures act like isolated events or have a cumulative effect; and if there is a cumulative effect, how it varies with the interval between exposures. The present report is concerned with exposures separated usually by intervals ranging from less

than a day to less than an hour, so that the opportunity for recovery is generally brief. A further exposure might aggravate a reversible effect that has not yet subsided, in which case the consequences of intermittent exposure might be similar to those of continuous exposure, even if the recovery intervals are longer than the exposure times. It is possible also that each exposure will add an increment of irreversible change that is undetectable after a single exposure but eventually becomes apparent. Other possibilities are that intermittent exposure may have more severe effects than continuous exposure and that an exposure may confer resistance against further exposure. The following discussion includes examples of all these possibilities.

Ehrlich[23] found that mortality in mice exposed to 0.5 ppm NO_2 before challenge with Klebsiella pneumoniae was enhanced over mortality in mice not pre-exposed, and that intermittent exposure (6 hours/day, 5 days/week, for 30 days) was more effective in this than continuous exposure (30 days). Ehrlich and Henry,[24] in similar experiments, found intermittent exposure (6 hours/day or 18 hours/day) somewhat less effective than continuous exposure. Pathological observations by Blair et al.[8] on the same animals showed that 24 hours/day, 7 days/week exposure was of similar or less effect in comparison with 6 hours/day or 18 hours/day, particularly as shown by alveolar enlargement. Gardner et al.[33] also studied potentiation of respiratory infection in mice and found intermittent exposure somewhat less effective than continuous exposure over the same period and at the same concentration. The intermittent exposures were made at 3.5 ppm NO_2 (15 daily exposures of 7 hours, 7 days/week) and 1.5 ppm NO_2 (21 similar exposures). Results were compared with controls continuously exposed at 3.5 and 1.5 ppm. At 3.5 ppm, continuous and intermittent exposure did not give significantly different results; at 1.5 ppm, continuous exposure was significantly more effective up to 14 days but not thereafter. However, the benefit of interruption was less than proportional to the reduction in exposure time.

Wagner et al.[95] exposed mice to 5 ppm or 25 ppm NO_2 for 7 weeks, and rats to 5 ppm for 56 weeks and then 25 ppm for 6 weeks. All groups were challenged at about the LCt_{50} level (approximately 70 ppm/5 hours). Mortality was heavy in all control groups, low or absent in previously exposed groups. Pathologic examination of rats after chronic exposure but not exposed to acute challenge showed frequent incidence of interstitial pneumonia, but this was seen also in control rats and cannot therefore account for modified response. (Mice were not examined after the conditioning exposure.) Hine et al.[50] also found that previous exposure of rats to NO_2 afforded some protection against subsequent exposure at levels causing 70 to 100 percent mortality in controls; however, they noted slowly resolving interstitial fibrosis in survivors of single acute exposures, so the protection was accompanied by respiratory impairment, possibly cumulative in repeated exposure.

Studies by Freeman and others have shown the development, in chronic low-level exposure of animals to NO_2, of lesions characteristic of irreversible lung damage (emphysema).[31,32] It may be argued that this is an accumulation of individually undetectable increments of tissue

destruction, which increments might also be accumulated in discontinuous exposure. This may be compared with the findings of Coffin, Gardner, and their associates on potentiation of respiratory infection by chronic low-level exposure of mice to NO_2. Mortality on bacterial challenge began to increase after 0.5 hours at 3.5 ppm, 2 hours at 1.5 ppm, and 120 hours at 0.5 ppm, and then increased linearly (vs. log time). Comparison of total dosages (Ct) shows that this is not a simple cumulative effect; concentration is the more important factor and so it takes disproportionately longer at low concentrations to build up the response. However, it does suggest an accumulation of damage to the lung or its defense mechanisms analogous to the buildup of emphysematous lesions in Freeman's studies.

The contrary evidence of protective action by a previous exposure in the work of Wagner et al.[95] and of others is not necessarily in conflict with the evidence of cumulative damage. Impairment of respiratory function, and particularly of respiratory intake and gas exchange, may well be protective against the lethal effects of intense exposure. The dose-response for lethality in NO_2 exposure is steep, with a difference of only twofold between LC_{90} and LC_{10}. A halving of the inhaled dose would effect a dramatic increase in survival, and might well be brought about by a combination of chronic respiratory deficiency and acute response to deep lung irritation with consequent airway constriction.

Differing opinions about repeated exposure have been expressed by various authorities. Cooper and Tabershaw[18] said that "intermittent exposures with intervening recovery periods are less harmful to experimental animals than continuus exposures." Tabershaw et al.[87] noted that NO_2 is self-protecting in single brief exposures and that there was "no evidence that NO_2 is a cumulative poison...(but) chronic effects may occur." Coffin,[14] commenting on the review by Tabershaw et al., said that "the most important aspect of NO_2 toxicity is its apparent cumulative effect" and cited Ehrlich[23] and the work of Freeman et al.[31,32] on emphysematous effects in chronically exposed rats. Mueller et al.,[65] discussing chronic exposure in relation to air pollution, said, "The suggestion is very strong that repeated exposure to NO_2 and O_3 can produce a cumulative effect."

As we have noted, the work of Coffin, Gardner, and their group provides a clear picture of accumulating lung damage in which there is a strong suggestion of a threshold concentration at which the latent period would be effectively infinite, and therefore of a slow "detoxification" process easily overcome by increasing dose rate. This evidence can be linked to intermittent exposure by the other experiments reported by Gardner et al.[33,34] and Coffin et al.[16] in which intermittent exposure of 7 hours/day at 1.5 ppm or 3.5 ppm NO_2 was not significantly less effective than continuous exposure (except over the initial 14 days at 1.5 ppm NO_2). The evidence is of effects after 7-hour exposure that are not repaired in 17-hour nonexposure.

A situation that may be encountered is superposition of short, high-level exposures on a continuous, relatively constant background.

This kind of exposure has attracted some attention in relation to air pollution, and it could arise from such things as a background of exhaust fumes containing NO_2 and intermittent peaks of propellant fumes from nitrocompounds. A study by Ehrlich et al.[25] compared the nonspecific immunological status of mice exposed (a) continuously to 2 ppm NO_2 or (b) continuously to 0.5 ppm with a daily peak of 1 hour at 2 ppm. The intermittent exposure showed significant ($p = 0.05$) changes in immunoglobulin levels (decrease in IgA vs. unexposed controls and increase in IgM, IgG_1, and IgG_2). The continuous exposure showed similar changes but less in every case and significant in only two of the four instances. This suggests that a safety margin should be allowed in estimating tolerable intensities for exposure in brief episodes where continuous background exposure is possible.

C. BRIEF EXPOSURE TO NITRIC OXIDE

Exposure to NO is discussed in a single section here because there is not enough evidence to support separate sections on human and animal exposure or a separate section on dose response. One reason for the evidence being sparse is the oxidation of NO to NO_2 by atmospheric O_2, which ensures that it is practically impossible to inhale pure (or nearly pure) NO in air by accident. In fact, it is not easy to avoid substantial contamination with NO_2 in carefully contrived laboratory experiments on inhaled NO. Consequently, response to NO is usually obscured or confused by the presence of NO_2, which is generally accepted to be the more aggressive toxicant by a substantial margin. However, this does not mean that the contribution of NO may be ignored, particularly if it is in greater concentration than NO_2 -- a likely circumstance in accidental exposure to NO_x from high-temperature zones. For example, Norwood et al.[70] reported accidental poisoning from working with an oxyacetylene torch in an unventilated space: in a reconstruction of the circumstances, analysis at 15 minutes gave 25 ppm NO_2 and 165 ppm NO; at 30 minutes, 90 ppm NO_2 and 180 ppm NO. Adley,[3] reporting a similar accident that was fatal to one worker, noted that the flame temperature was approximately that used in commercial production of NO. The rate of oxidation varies with the square of the NO concentration (e.g., 50 percent oxidation to NO_2 in 40 minutes at 100 ppm NO; 50 percent oxidation to NO_2 in 420 minutes at 10 ppm NO). It follows that a substantial proportion of NO_2 is to be expected unless the concentration of NO is low and the time since mixing with air is brief.

The early work of Pflesser (1935)[77] has been quoted many times, but it is questionable what composition of NO_x his mice were exposed to and his dose-response slope is very difficult to believe; he reports an LC_{50} (of unspecified duration) of 320 ppm NO and an 8-hour LC_0 of 310 ppm. He also found NO more toxic than NO_2 (LC_{50} of 1500 ppm NO_2), an observation that is unsupported by other evidence except for Paribok's similar finding of greater toxicity for NO in 1 hour exposure but not in 6 to 8 hours exposure.[22] In contrast to his quantitative data,

Pflesser's descriptions fit very well with current ideas about the toxicology of the two gases: mice exposed to NO showed severe cyanosis, no edema, brown blood (not analytically confirmed as methemoglobinemia), and rapidly reversible response if rescued in time; NO_2 mice showed immediate irritation, more delayed and less severe cyanosis, edema, deep red blood, and slow recovery from nonfatal exposure.

There are many reports of accidental exposure to NO_x in one form or another, and these are variously described as nitrous fumes, nitrogen oxides, or nitrogen dioxide; there is no doubt that these exposures often involved large proportions of NO, but the evidence is very sketchy. We have cited Norwood[70] and Adley,[3] and the reconstruction in the former study showed NO in substantial excess. Clutton-Brock[54] reported two accidental exposures to N_2O contaminated with NO in surgical anesthesia, one fatal. There must have been considerable oxidation to NO_2 when the gas was mixed at 15,000 ppm with 25 percent O_2 and later 50 percent O_2. These accidents, and animal experiments arising from them, were at very high concentrations, in the range of 5,000 ppm to 20,000 ppm. They are of limited interest here, partly because of the high concentrations themselves and partly because experiments at these concentrations necessarily mean considerable and rapid oxidation of NO to NO_2 in exposures designed to be to NO only; in fact, one paper reports a clearly visible red-brown color in the mixed gas. However, it is of interest that Greenbaum et al.[41] attributed death of exposed dogs to critical reduction in arterial oxygen content caused in part but not entirely by methemoglobinemia, which arises in NO_2 exposure but is more characteristic of NO exposure.

Reports on various aspects of the hematologic effects of NO_x intoxication present an incomplete and inconsistent picture. The nature of the possible interactions is not the main issue; there is general agreement with, e.g., Kon et al.,[54] who conclude that NO diminishes O_2 transport by human erythrocytes through three mechanisms: (1) tight bonding of NO to hemoglobin (Hb), forming NOHb; (2) increased affinity of Hb for O_2; and (3) formation of methemoglobin (MetHb). It is probable that MetHb is formed from NOHb by oxidation and that NO-MetHb may also be an intermediate. The issue is the quantitative one: the affinity of Hb for NO in vitro is extremely high (very much more than for CO), but Oda et al.[71] found a plateau of only 0.13 percent NOHb (as percent of Hb) in animal exposures. It is generally believed that this is due to the presence of O_2, which is absent in anaerobic in vitro tests. MetHb has been found in man, e.g., after accidental exposure for a few minutes to NO_2 at several hundred ppm,[46] but at low levels and it seems that conversions sufficient to cause serious hypoxia can only result from extraordinarily intense exposures. The work of Toothill[88] shows that a dog exposed at 20,000 ppm NO reached 5.3 percent MetHb in 4 minutes and approximately 100 percent in 50 minutes. (He did not detect NOHb or NO-MetHb.) At 1000 ppm NO_2, MetHb (at 4.6 percent) and visible cyanosis were not observed until 155 minutes.

Some experiments have been done with animals and volunteers at much lower concentrations. The work of Oda et al.,[71] previously cited, was with mice, rats, and rabbits exposed to 10.6 ppm NO and 0.8 ppm NO_2;

the NO-air mixture was passed through soda lime to remove NO_2, but the analysis shows that this was not very effective in excluding NO. Oda does not report any consequences of exposure other than the formation of up to 0.13 percent NOHb, but his team also made the interesting observation that mice exposed to 12.8 ppm NO_2 showed the same NOHb spectrum and almost the same electron spin resonance intensity as those exposed mainly to NO; since the NO_2 was from a cylinder of 1 percent NO_2 in air, this is clear evidence of NOHb formation from NO_2 alone (and hence of possible formation of MetHb from NO_2). It should be noted that the mechanism of this could involve intermediary formation of NO from NO_2.

Von Nieding et al.[94] exposed volunteers to NO; the test subjects were patients with chronic respiratory disease, and healthy controls were included. The NO-air mixture was passed through alkalinized salicylic and sulfanilic acid to remove NO_2 and through a filter to remove any aerosol formed from the wash liquid, but no analysis is reported to show how effective the NO_2 removal was. Exposures to NO_2 were also reported from earlier work. The most significant findings (in bronchitics) are shown in Table 2.

Table 2. Physiological Effects in Volunteers Exposed to NO_2 or NO

Effect	Threshold Exposure for Effect
Decrease in arterial partial pressure of O_2	NO_2: 5 ppm/15 minutes NO : above 1 ppm
Decrease in lung diffusion capacity for CO	NO_2: 5 ppm/15 minutes NO : none at up to 39 ppm
Increase in airway resistance	NO_2: 1.6 to 2.0 ppm NO : above 20 ppm

The results show NO is considerably less toxic than NO_2 but not without effect. It is to be noted that the physiological responses may be regarded as an index of acute primary irritancy, which has two possible implications: (1) that they result, totally or in part, from NO_2 irritancy and (2) that the tests missed a significant nonirritant effect of NO, masked by the irritant response.

Hugod[51] exposed rabbits to 43 ppm NO plus 3.6 ppm NO_2. He used both a salicylic/sulfanilic NO_2 trap as von Nieding[94] did and a soda lime trap as in Oda's work.[71] Exposure was for 6 days and no significant change was seen in the lungs, in contrast to his earlier finding of positive response to 14 days exposure at 5 ppm (and similar observations by others). No fully satisfactory explanation is available for the discrepancy, but the study suggests that 43 ppm NO is not particularly harmful to lung tissue in subchronic exposure.

Azoulay et al.[6] exposed rats to 2 ppm NO for 6 weeks. Control of NO_2 was very effective, the concentration (including atmospheric background) not exceeding 0.08 ppm; this may probably be attributed in part to the low NO concentration and hence low oxidation rate. The exposure was without significant observed effect; no MetHb was detected and there were no histopathological signs.

There is clearly insufficient evidence for development of a dose-response model for humans or even a firm definition of the health effects of NO. However, in the context of short, high-level exposures, it is likely that there will always be significant amounts of NO_2 accompanying the NO and the problem can therefore be redefined as the determination of what additional contribution NO may make. Additional effects of NO may include "irritant response" of bronchiolar-alveolar tissues if von Nieding's results were in fact due to NO and not to NO_2 contamination, but it is unlikely that there will be any persistent damage if Hugod's negative observations at 43 ppm NO/6 days in rabbits are sound.

It is reasonable to expect a considerably less intense response to NO than to NO_2 but nevertheless a significant one. In 1976, NIOSH supported retention of the federal standard for NO of 25 ppm TWA and cited the German Democratic Republic's maximum allowable NO concentration of 16 ppm; at that time, the standard for NO_2 in both countries was 5 ppm.[69]

D. EXPOSURE TO NO_x TOGETHER WITH OTHER FACTORS

1. NO_x with Other Toxicants

Two of the other toxicants studied in this program have been tested in admixture with NO_2: SO_2 and CO. Abe[1] reports exposure of five healthy adult males to NO_2 or SO_2 at 4 to 5 ppm or to a mixture at 2.5 ppm each. Effective lung compliance decreased and an increase was observed in inspiratory and expiratory resistance, either during the 10-minute exposure or later. The results clearly show independent action of the two gases: SO_2 elicits an immediate response and rapid recovery after exposure; the effects of NO_2 set in towards the end of exposure or later; and the mixed gases display a clear compromise between the two regimes. It can be confidently stated that NO_2 and SO_2 are simply additive at these concentrations as far as acute and reversible respiratory response is concerned. DiPasquale et al.[21] studied the effect of CO at concentrations giving about 25 percent COHb on the LC_{50} ofNO_2 for mice and rats in 5-minute exposure. The results (Table 3) show a statistically significant difference between species but not between exposure with and without CO. This is evidence against any likely effect of CO on exposure to NO_x at lethal concentrations, but not necessarily in the case of other sublethal responses. It is likely, for example, that the tissue hypoxia induced by carboxyhemoglobinemia would additively enhance hypoxia induced by various effects of NO_x on respiratory gas exchange, oxygen-carrying ability of the blood, and

cellular respiratory enzymes (all discussed elsewhere in this report). Hine et al.[50] found that concurrent exposure to CO_2 at 5 percent (50,000 ppm) increased the lethality to rats of NO_2, but only at the higher NO_2 concentrations tested.

Table 3. Influence of CO on Lethality of NO_2

		LC_{50} (95 percent limits) for 5-minute exposure (ppm)
Mice	NO_2	1880 (1345-2626)
	NO_2 + CO	1644 (1203-2247)
Rats	NO_2	831 (556-1240)
	NO_2 + CO	1140 (720-1707)

2. NO_x with Physical Stresses and Nontoxicants

Hine et al.[50] measured lethality of NO_2 in rats exposed to cold (4° C for 72 hours before NO_2 exposure), heat (100° F for 24 hours before NO_2 exposure), and extreme exercise (swimming to near exhaustion, followed by (a) re-exercise before exposure and (b) exposure before re-exercise). Hine's results show increased lethality after exposure to cold; not after heat; and after postexposure exercise but not preexposure. The point is that violent exertion after NO_2 exposure is shown to affect response. While such intense exposures are outside the likely range to which the study applies, the interaction of exposure and exertion does suggest that hypoxia induced by pulmonary edema and increased airway resistance (both of which occur at very much lower levels of exposure) may significantly reduce the maximum level of exertion in humans affected by NO_x.

There is some evidence of potentiation of NO_2 effects by concurrent exposure to an otherwise ineffective aerosol. Nakamura[66] found that exposure to NO_2 with an aerosol of NaCl caused greater increase in airway resistance than NO_2 alone, in line with several observations on a variety of irritant gases, and there is little doubt that concurrent exposure to "respirable" particles is likely to aggravate the immediate and reversible effects of NO_2 on respiratory mechanics.

E. SPECIES SENSITIVITY

Interspecies comparisons between different investigators and laboratories may be questionable because of differences in technique. Fortunately, some comparisons can be made within sets of experiments at one laboratory, notably the work of Hine et al.[50] Taking the response of

the rat to lethal concentrations as standard (relative sensitivity 1), mice and dogs can be shown to be similar (relative sensitivity 1.2 to 1.4) and guinea pigs somewhat more sensitive (1.9). Rabbits were less sensitive, but the data are too scattered for analysis. Carson et al.[12] found qualitatively less response in dogs than in rats when exposed to NO_2 at approximately 50 percent and 25 percent of the rat LC_{50}. DiPasquale et al.[21] found mice more resistant than rats: the 5-minute LC_{50} for rats was 831 ppm, for mice 1880 ppm. Hilado et al.[49] found Swiss Webster mice more sensitive (10-minute LC_{50} was 1000 ppm) than ICR mice (10-minute LC_{50} was above 2000 ppm), but the experimental technique and reporting of data are not good. Ehrlich[23] found some strains of mice less sensitive to infection enhancement by prior exposure to NO_2 (strains BDF_1 and LAF_1) or postexposure (strain $C_{57}BL/c$), but concluded that NO_2 damage was not closely related to strain differences.

Gray et al.[49] found significant differences between old and young rats of the same strain in survival time when exposed to NO_2 at about 250 ppm; the old rats survived longer than the young, significantly so in the sets male/female, female/male, and female/female, but not in male/male.

F. CONCENTRATION-TIME RELATIONSHIPS

1. Previous Observations

Gray et al.[38] found that concentrations of NO_2 causing 50 percent mortality in rats (LC_{50} values) were related to time by the expression $LC_{50} = 2051/t^{0.609}$. This is a form of the general expression $Ct^n = K$, in which C is the concentration, t is the time of exposure, n is a constant for the given toxicant and species of animal, and K is constant for a given level of effect for that toxicant and species. Data satisfying this equation lie on a straight line if plotted against log C and log t. The exponent of time, n, determines the slope of the line. If n = 1, the line is at 45° to the log C and log t axes; as n becomes smaller, the line approaches parallelism with the time axis. The constant K determines the distance of the line from the origin and it varies directly with the concentration required to elicit a given effect in a given time. The expression $Ct^n = K$ has been found applicable to many inhaled toxicants.

Carson et al.[12] obtained LC_{50} data for exposure of rats to NO_2 and found that the data lay on a straight line when LC_{50} was plotted against log t. The investigators used a graphical extrapolation of parallel lines to express lower (sublethal) levels of effect but did not calculate the time exponent, n, which can be shown to be approximately 0.52.

The American Industrial Hygiene Association[5] used the data of Carson et al. in estimating occupational emergency exposure limits,

applying the model $Ct^n = K$ to data from Meyers and Hine[63] on exposure of volunteers to acutely irritant concentrations of NO_2. The method was not explicitly described, but it can be shown that the recommended limits for 5, 10 and 30 minutes exposure form a straight log-log plot and give the value of 0.312 for n. The emergency limit for 60 minutes is given as 10 ppm, which is inconsistent with the others; 15 ppm would fit the line closely.

Larsen et al.[55] analyzed data from Gardner et al.[34] on the potentiation of respiratory infection in mice by exposure to NO_2 before exposure to a respiratory pathogen. Concentrations ranged from 0.5 to 28 ppm NO_2 and exposure times from 6 minutes to 1 year. These data fitted the model $Ct^n = K$ and n was calculated to be 0.33.

G. CONCLUSIONS

1. Nitrogen Dioxide

The direct biologic effects of NO_2 exposure occur almost entirely at the surfaces of the respiratory tract and other mucosa. The effects result from immediate primary irritation and delayed tissue damage in the lung. Secondary effects at other sites may result from hypoxia caused by impaired ventilation and there may also be some impairment of oxygen uptake by hemoglobin and of cellular respiration. There is also some evidence of biochemical and histologic changes in other organs and of immunologic changes that might be direct effects of NO_2 or mediated by lung damage through release of antigenic material.[25]

The consequences of the most intense exposures Listed in descending order of exposure intensity are:

Immediate death from respiratory spasm and asphyxia

Early death from pulmonary edema

Delayed death or incapacitation from edema, bronchiolitis obliterans, or infective pneumonia

Immediate and reversible incapacitation from respiratory irritation.

Survivors usually show complete remission, but there is some evidence from animal experiments that tissue changes after a single response may be irreversible or slow in resolution. In less intense exposures, edema and bronchiolitis obliterans may be asymptomatic or subside without clinical intervention. It is unlikely in the context of this study that exposures intense enough to cause immediate discomfort or more serious effects would be repeated.

Animal experiments on enhanced respiratory infection that have been used to study effects of low NO_2 concentrations also provide evidence

of a possible effect on respiratory infection in man. The experiments have included exposure to NO_2 either before or after bacterial challenge and (within certain limits) both sequences give enhanced infection. It is also established that single brief exposure can induce the effect--1 hour at 3.5 ppm NO_2 and only 6 minutes at 28 ppm. It appears likely that a similar effect could occur in man, but it has not been proven. However, it must be noted that the bacterial challenge is with a dose-causing mortality in animals not exposed to NO_2. There is no evidence that enhancement of infection occurs in animals naturally infected and exposed at those low levels of NO_2 concentrations. Subsequent infection is seen only in survivors of intense exposures, as a sequel to chemical pneumonitis.

The other major body of data from low-level exposures is that on respiratory impairment and other acute and reversible responses, mainly in volunteers but including some animal experiments. The studies of von Nieding and others show that brief exposures to NO_2 at 5 ppm and even lower concentrations induce measurable deterioration of respiratory functions such as airway resistance, lung compliance, diffusion capacity, and arterial partial pressure of oxygen. The general picture is one of impairment to ventilation, gas exchange, and oxygen transport that could induce significant hypoxia. There is, however, no clear evidence that it is likely to be significant in the context of the present study. A consequence of potential importance is impaired work capacity and physical endurance. It seems unlikely that this would be apparent in short-term efforts drawing on the tissues' oxygen reserves, but when these were depleted a significant deterioration would be expected. Unfortunately, there are no suitable data on volunteers exposed to NO_2. Attempts to argue from other conditions would be suspect because of differences in, for example, the regional effects of other toxicants on the lung. The conclusions to be drawn are that there is no present basis for estimating the significance of NO_2-induced hypoxia, that the effect could be significant, and that it could be tested in volunteer exposures of the type conducted by von Nieding and others.

The evidence on effects of repeated exposure suggests that relatively brief intervals between exposures (less than 24 hours) are generally insufficient for complete recovery. Either a reversible effect fails to subside or there is an accumulation of irreversible damage. The evidence from infection-enhancement experiments is that intermittent exposure is somewhat less effective in enhancing mortality than continuous exposure over the same period and at the same concentration. It appears reasonable to expect no benefit from resting periods between same-day exposures. It has been suggested earlier that evidence of cumulative damage is also relevant to intermittent exposure; it is seen in chronic exposure of animals with slow development of emphysematous lesions, in the delayed response of animals to infection enhancement, in slowly resolving interstitial fibrosis after single acute exposure, and in other observations.

2. Nitric Oxide

NO is less toxic than NO_2; it is far less irritating and less able to cause tissue damage. Occupational standards favor an approximation of fivefold less toxicity than NO_2, i.e., the NO concentration for a given exposure time and response would be five times the NO_2 concentration for the same time and response.

3. Suggested Approach to Developing Criteria

The American Industrial Hygiene Association,[5] in a report on emergency exposure limits for NO_2, assumed single exposures and the applicability of the $Ct^n = K$ model. The AIHA used animal data from lethal and sublethal exposures (down to approximately 25 percent of LC_{50}) together with unpublished data from Hine on volunteer exposures to arrive at estimates of exposures that may involve acute discomfort but will not prevent self-rescue.

For combined exposure to NO_2 and other irritants such as SO_2, it is suggested that the simple additive model be used, i.e., each component exposure is expressed as a simple fraction of its ceiling and the sum of the fractions should not exceed unity. It is recognized that this may overestimate the contribution of a rapidly reversible irritant such as SO_2.

It is not possible at present to make estimates for single or repeated exposures at levels below those causing immediate discomfort because there is no suitable dose-response data base. The principal concern here is that respiratory impairment might induce sufficient tissue hypoxia to cause significant deterioration in work performance, endurance, sensory responses or mental alertness. There are insufficient data to support even semiquantitative estimates of dose response.

The second concern in exposures below the level of acute harassment is cumulative damage, irreversible or only slowly reversible and primarily in the alveolar and distal bronchiolar region of the lung. There is evidence of persistent damage in humans but only after intense exposure. Animal data from lower levels of exposure show evidence of irreversible changes after chronic or repeated exposures, and there are some indications of precursors of persistent damage after single, relatively brief exposures. However, there is no validated basis for extrapolating the animal data to man.

A further possibility is enhanced respiratory infection. As already noted, there is no evidence at present about the direct applicability of the infectivity model, based on intense challenge with respiratory pathogens, to humans exposed only to normal risks of respiratory infection. Epidemiological data from populations exposed to elevated NO_2 levels suggest enhancement of respiratory infection, but multifactorial exposure to other pollutants including irritants obscures the causal relationship and precludes conclusions about the influence of NO_2.

V. SUGGESTED FOLLOW-ON WORK

The following suggestions for follow-on work are directed principally towards evaluation of the practical significance of reversible respiratory impairment and further investigation of intermittent exposure, including recovery after acute exposure.

A. RECOVERY FROM ACUTE EXPOSURE

The rate and completeness of recovery after a single acute exposure have not been adequately studied. This information is important to evaluation of single and intermittent exposures. Data from animals at various levels of concentration in acute exposure up to 1 hr would support a better understanding of the practical consequences of single exposure and of further exposure during a recovery period. They would also permit a much more thorough analysis of the consequences of repeated exposure as far as reversible effects are concerned.

B. RESPONSES IN INTERMITTENT EXPOSURE

The present evaluation of intermittent exposure depends heavily on data from infectivity enhancement. More data on other responses, and especially those related to lung damage such as the histopathological findings of Blair et al.,[8] would strengthen the analysis.

C. EFFECT OF PEAK EXPOSURES ABOVE A BACKGROUND

Evidence of Ehrlich et al.[25] is that brief peaks of 2 ppm NO_2 above a continuous 0.5-ppm background are more effective in inducing changes than continuous exposure at 2 ppm. The indicator of response was a change in immunoglobulin levels that may have been caused by antigenic material released by lung tissue damage, but this is not proven. Confirmation with other indicators, and especially direct indicators of lung damage, would be a most important finding.

D. EFFECT OF COFACTORS

A positive influence of physical stresses (cold, exertion) has been shown in animals, and NO_2 is additive to one of the other toxicants studied in this project, SO_2. There is some evidence that inert aerosols aggravate the effects of NO_2 on respiratory function. These kinds of interactions could be usefully studied further with special reference to known stresses and possible coagents in the military context such as screening smokes.

VI. REFERENCES

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* Abstract in Literature Review (Appendix)

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APPENDIX A

REVIEW OF THE LITERATURE

1. Exposure to NO_2

A. Human

• Hatton DV, Leach CS, Nicogossian AE, Ferrante ND: Collagen breakdown and nitrogen dioxide inhalation. Arch Environ Health 32:33-36, 1977

Review:

Three astronauts were accidentally exposed to NO_2 during the descent phase of the Apollo-Soyuz mission. The description of the incident and estimates of exposure intensity and time are quoted verbatim in the following review because they provide the only data on exposure in the paper.

"Because of inadvertent firing of the Reaction Control System (RCS), the NO_2 entered the command module through the cabin pressure relief valve, which was opened during the landing sequence. During postlanding debriefing, the Apollo commander reported the appearance of a thick, yellow brown smoke, which cleared very rapidly. The RCS was immediately turned off, fresh air was drawn into the cabin, and the NO_2 vapors were absorbed by the lithium hydroxide canisters; nevertheless, the crew's exposure to the gas lasted 4 minutes and 40 seconds. Postflight analyses of the lithium hydroxide canisters and visual comparison of different color shades of NO_2-air mixtures suggested a peak cabin concentration of 750 ppm NO_2 at 1 atmosphere (1530 mg/m^3), and an average exposure to 250 ppm of gas (510 mg/m^3)."

Symptoms 1 hour after exposure included tightness of the chest, retrosternal burning sensation, inability to inhale deeply, and nonproductive cough. The symptoms worsened the next day and the subjects were unable to hold their breath or perform the forced expiratory maneuvers required for pulmonary function tests. X-rays suggested alveolar exudation characteristic of chemical pneumonitis and blood gas analyses indicated mild alkalosis and hypoxia with hyperventilation. The subjects were asymptomatic on the third day and chest X-rays were normal on the fifth day after exposure. Another measurement that showed some change was the methemoglobin level, which, in an early post-exposure measurement, showed a 4.2 percent increase over the pre-exposure level; this decreased to 2 percent on the next day.

Urine samples taken soon after exposure showed significantly higher amounts of hydroxylysine glucosides. The authors cite work that suggests that "the urinary excretion of various forms of hydroxylysine may represent an index of collagen breakdown." This, coupled with the fact that 60 to 80 percent of the dry weight of a normal lung is made up by collagen spread over a large surface, leads the authors to suggest that

"the increased urinary excretion of hydroxylysine metabolites might have been related to the acute pulmonary lesions caused by the inhalation of the toxic gas," i.e., NO_2.

Analysis:

The estimates of exposure intensity are questionable because they are out of line with other estimates of human exposure for comparable response and many experimental exposures of animals, in that an average exposure of 250 ppm NO_2 for 4.7 minutes with a peak of 750 ppm would be expected to cause fatalities or at least very severe and immediate incapacitation and long hospitalization. At the estimated peak of 750 ppm NO_2, a few breaths would be dangerous. Possible explanations of the discrepancy are:

1. The time of exposure was overestimated. (The method of estimation is not stated.)

2. The subjects held their breath, especially in the initial and presumably most intense exposure.

3. The peak concentration was presumably estimated from recollections of color intensity which is a poor estimate of concentration.

4. The average concentration was presumably estimated from analysis of the lithium hydroxide canisters and calculation of the total amount of NO_2 absorbed. If much of the NO_2 bypassed the subjects' breathing zone through incomplete mixing, the average concentration they experienced would be overestimated.

The NO_2 was presumably commercial grade containing small concentrations of NOCl, NO, and other impurities. The reported symptoms are consistent with exposure to a high sublethal dosage of NO_2 and the evidence of collagen breakdown is consistent with observations in animal experiments.

• Von Nieding G, Wagner M, Krekeler H, Smidt U, Muysers K: Grenzwertbestimmung der akuten NO_2-Wirkung auf den respiratorischen Gasaustausch und die Atemwegswiderstande des chronisch lungenkranken Menschen. Int Arch Arbeitsmed 27:338-348, 1971

• Von Nieding G, Krekeler H: Pharmakologische Beeinflussung der akuten NO_2-Wirkung auf die Lungenfunktion von Gesunden und Kranken mit einer chronischen Bronchitis. Int Arch Arbeitsmed 29:55-63, 1971

• Von Nieding G, Krekeler H, Fuchs R, Wagner M, Koppenhagen K: Studies of the acute effects of NO_2 on lung function: influence on diffusion, perfusion and ventilation in the lungs. Int Arch Arbeitsmed 31:61-72, 1973 (See also von Nieding et al. (1975), reviewed under "Nitric Oxide," which describes experiments with NO_2 as well as NO.)

Review:

Studies were made of the acute effects of NO_2 exposure on respiratory functions in healthy subjects and patients with chronic nonspecific lung disease (bronchitics).

Volunteers were exposed to NO_2-air mixtures from a polyvinyl-chloride bag via a flap valve with 50-ml dead space. NO_2 concentrations were measured by Saltzman's method.[79] Respiratory functions were measured before, during, and after exposure to NO_2. Airway resistance was measured by whole body plethysmography. Tidal volume, frequency, and minute volume were recorded. Respiratory gases were measured by mass spectrometry (Varian) or infrared absorption. Arterial blood was obtained from the hyperemized ear lobe and analyzed by polarography or potentiometry; pH was measured by microelectrode. The differences between alveolar (end-expiratory) and arterial partial pressures of O_2 and CO_2 were recorded.

In the first series of tests, 25 bronchitics inhaled for 15 minutes at 2, 4, or 5 ppm NO_2, and 63 bronchitics inhaled 30 breaths at 0.5 to 5 ppm NO_2. Alveolar partial pressure of O_2 was not significantly affected. Arterial partial pressure decreased significantly ($p < 0.02$) at 4 or 5 ppm, but not at 2 ppm. Airway resistance increased significantly at 2.1 to 2.5 ppm ($p = 0.01$) and at 1.6 to 2.0 ppm ($0.1 > p < 0.05$), but not at 1.5 ppm and below.

In the second series of tests, 14 healthy subjects and 23 bronchitics were exposed to NO_2 at 5 to 9 ppm for 30 breaths or for 15 minutes, before and after treatment with an anticholinergic (atropine), a sympathomimetic (orciprenaline), and an antihistaminic (meclastine). The antihistaminic drug protected against increased airway resistance and decreased gas exchange; the other drugs did not. The authors concluded that NO_2 acts by release of histamine causing bronchiolar, alveolar, and interstitial edema.

In the third series of tests, 55 healthy subjects and 84 bronchitics were exposed:

In 16 healthy subjects, CO diffusing capacity was measured after 15 minutes of exposure to 5 ppm NO_2.

In 14 bronchitics, respiratory O_2 and CO_2 gas exchange was measured before, during ,and after 60 minutes of exposure to 5 ppm NO_2.

In 39 healthy and 70 bronchitic subjects, airway resistance was measured before and after 30 breaths of NO_2 at 0.5 to 5 ppm.

CO diffusing capacity was measured by inhalation of 20.9 percent O_2, 0.3 percent CO, and 4 percent He in N_2, by the single-breath method of Krogh as modified by Ogilvie.

Lung perfusion was studied in rabbits exposed to 30, 40, or 50 ppm NO_2 for 15 minutes. Radiolabeled albumin was injected into an ear vein and distribution was observed by scintigraphy. ^{99m}Tc was used before and ^{131}I after exposure to NO_2, and they were differentiated by their different gamma radiation spectra.

Data were analyzed by the paired ranking method of Wilcoxon. Results showed a significant decrease in CO diffusing capacity ($p < 0.01$) in the 16 healthy subjects after 15 minutes at 5 ppm NO_2. There was no signifi- cant difference between 9 nonsmokers and 7 smokers. Gas exchange was impaired, as shown by decreased arterial O_2 partial pressure, after 15 minutes exposure of bronchitics at 5 ppm; no further change took place until exposure was discontinued at 60 minutes. Increased airway resistance was observed in bronchitics at 1.6 ppm NO_2 (30 breaths) and above, and it correlated with the intensity of exposure ($r = 0.542$); no effect was observed at or below 1.5 ppm NO_2, or in healthy subjects at any level.

The perfusion experiments showed centralization of the radiotracer after exposure to 30 ppm NO_2 for 15 minutes, and more markedly after 40 or 50 ppm NO_2.

The authors concluded that the change in perfusion could be an adaptation to changed ventilation or an effect of edema. Taking account also of other animal experiments, they concluded that impairment of pulmonary function after acute inhalation of low concentrations of NO_2, if frequently repeated, might influence the development of chronic bronchitis and emphysema.

Analysis:

The reported NO_2 exposures are of acceptable reliability for the present study, bearing in mind the analytical method used and the consistency of responses at very low concentrations. However, it would have been better to know more about the origin of the NO_2 and the variance among nominally identical samples.

The physiological techniques used by von Nieding have been criticized for not taking full advantage of state-of-the-art methods and also on questions of personal preference. For example, blood sampling by arterial catheter is less open to error than ear lobe puncture, but the latter--if performed to preparatory warming to encourage blood flow--can be reliable and is more convenient and acceptable for the experimenter and subject. Similarly, whole body plethysmography is more prone to error than, for example, a mouthpiece flowmeter and esophageal balloon for volume, rate, and pressure measurement, but it is reliable in the hands of a good technician. A third point is that some investigators would prefer use of pure O_2 inhalation rather than end-expiratory gas analysis for O_2 exchange measurement and for closing volume measurements. The consensus, however, is that von Nieding's results are reliable.

The statistical technique of Wilcoxon[97] is appropriate for small samples and responses of unknown statistical distribution because of its nonparametric approach.

The conclusions regarding the mechanism of NO_2 action are consistent with the evidence and many other observations. It is notable that the body shows a quick response, which does not change thereafter (within the period of these tests). The authors' conclusion about long-term and possibly irreversible lung damage from frequent acute exposures to low concentration is supported by the evidence of others rather than their own.

• Abe M: Effects of mixed NO_2-SO_2 gas on human pulmonary functions. Bull Tokyo Med Dent Univ 14:415-433, 1967

Review:

The subjects were 5 healthy adult males with no history of respiratory disease, 4 nonsmokers and 1 light smoker. They were exposed successively to NO_2 (4-5 ppm), SO_2 (4-5 ppm), and NO_2 + SO_2 (2.5 ppm each) at 2-week intervals.

Commercial 99.5 percent NO_2 was injected into a 140-liter vinyl bag and diluted with metered air. NO_2 concentration was measured by Saltzman's[79] method and confirmed immediately before inhalation by Kitagawa-type detection tube. Seated subjects breathed the gas mixture through a valved mouthpiece for 10 minutes while wearing nose clips. They then breathed room air for 30 minutes. Measurements of effective compliance, inspiratory resistance, and expiratory resistance were made before exposure, immediately after exposure, and 10, 20, and 30 minutes after exposure. Spirometric measurements were made before and 30 minutes after exposure, using a Benedict-Roth spirometer and Wright Peak Flow Meter. The other measurements were made with a mouthpiece differential transducer flowmeter and esophageal balloon.

Exposures to SO_2 and NO_2 + SO_2 were made in the same manner.

Effects of NO_2 began to be seen at the end of exposure or 10 minutes later, then increased linearly. When the last measurements were made at 30 minutes after exposure, effective compliance was 59 percent of initial value, inspiratory resistance 192 percent, and expiratory resistance 172 percent. Spirometer readings showed no change.

In contrast, SO_2 showed maximal increase in resistance at the end of exposure and recovery in 10 to 20 minutes; there was no marked change in compliance and no change in spirometric measurements. The mixed gases showed intermediate responses.

The author comments that his results with SO_2 are similar to those of Frank et al.[30], Yokoyama,[99] and Suzuki and Ishikawa.[85] He attributes the change in compliance with NO_2 but not with SO_2 to

action, respectively, on the deep lung and upper respiratory tract, in accordance with Patty's[23] explanation that SO_2 dissolves and reacts immediately in the wet mucous surface of the upper tract while NO_2 reaches the alveoli and reacts slowly, tending to cause pulmonary edema. He comments that the increasing changes at 30 minutes after exposure suggest a long-remaining aftermath.

Analysis:

The NO_2 concentrations were prepared and measured by acceptable methods, and the physiological measurements should also be reliable.

Although Abe comments that the measurements at 30 minutes after exposure suggest a long-remaining aftermath he does not say why no later measurements were made. (These readings are often described incorrectly as maxima, e.g., by NIOSH,[69] National Academy of Sciences,[68] and World Health Organization.[98])

The difference between SO_2 and NO_2 could also be explained by SO_2 acting directly on the parasympathetic nervous system and NO_2 acting indirectly through stimulation of histamine release.

Abe does not comment on the absence of spirometric response. A paper published in the same year (1967) by Macklem and Mead[59] describes experiments in which they used a retrograde catheter to measure pressure within the airways and found the forced expiratory volume a very insensitive measure of airway constriction.

• Mangold CA, Beckett RR: Combined occupational exposure of silver brazers to cadmium oxide, nitrogen dioxide and fluoride at naval shipyard. Am Ind Hyg Assoc J 32:115-118, 1971

Two silver brazers working in a poorly ventilated space, who discontinued work after 30 minutes because of respiratory irritation, were treated and were hospitalized 6 to 8 hours later with acute pulmonary edema. Both experienced lung damage and one retired on disability. The authors present evidence that cadmium oxide was not involved and fluoride was probably not significant. Reconstruction of the event, with sampling for NO_2 (by unspecified method), showed that NO_2 quickly increased to 50 ppm and then to 122 ppm in 30 minutes.

• Norwood WD, Wisehart DE, Earl CA, Adley FE, Anderson DE: Nitrogen dioxide poisoning due to metal-cutting with oxyacetylene torch. J Occup Med 8:301-306, 1966

Two men worked with an oxyacetylene torch in an unventilated space. One worker left after 15 minutes and experienced cough and shortness of breath. Another remained for 30 minutes, experienced respiratory discomfort, temporarily ventilated the space, and returned to finish the job in

15 minutes. This worker had considerable breathing difficulty overnight and was seen by a physician about 18 hours after exposure; forced expiratory volume was normal, vital capacity about 50 percent, and white blood cell count elevated. He was hospitalized for 7 days. Pulmonary edema was diagnosed. Recovery was uneventful and examination at 73 days suggested no chronic effect.

The accident was reconstructed by a worker (with respiratory protection) in a similar space for 45 minutes. The following data are from the table of results (all other measurements were of NO_x total only):

Time, min	NO_2, ppm	NO, ppm
15	25	165
30	90	180

The authors note that the relative increase of NO_2 is consistent with slow oxidation of NO, the rate of which is stated to be 11 ppm NO_2/minute at 200 ppm NO and 2.8 ppm/minute at 100 ppm.

• Adley FE: Exposures to oxide of nitrogen accompanying shrinking operations. J Ind Hyg Toxicol 28:17-20, 1946

Four workers present during a "shrinking" operation (heating a steel plate by oxyacetylene flame) in a small unventilated space showed respiratory symptoms, and one died 10 days later from chemical pneumonia. Adley reconstructed the conditions of exposure to measure NO_x exposures; samples were oxidized in H_2SO_4-H_2O_2 on collection. Eight samples over a period of 23 minutes gave an average concentration of 196 ppm NO_x with a maximum of 480 ppm at 8 minutes. Adley made observations on himself, which included the following:

Time, min	NO_x(as NO_2), ppm	Effects
1	210	Cough, tightness of chest
2	270	
3	320	
4	320	
1	240	Cough, tightness of chest
4	370	
5	430	
7	370	

He noted that the flame temperature "slightly below 6000° F was approximately that used in commercial production of NO," and concluded that his NO_x probably contained a substantial proportion of NO.

• Meyers FH, Hine CH: Some experiences of NO_2 in animals and man. Paper presented at the Fifth Air Pollution Medical Research Conference, Los Angeles, 1961

Young adult male volunteers were exposed to NO_2 at 50 ppm and 25 ppm. Within 1 minute at 50 ppm, 2 out of 7 subjects left the chamber complaining of severe substernal pain. About half of the subjects found 5 minutes of exposure at 25 ppm unpleasant but tolerable.

• Lehmann KB, Hasegawa: Studies on the effects of technically and hygienically important gases and vapors on man (31)--The nitrous gases--Nitric oxide, nitrogen dioxide, nitrous acid, nitric acid. Arch Hyg 77:323-368, 1913

NO_x formed by reaction of nitric acid and copper was introduced in measured volume into an 11-m^3 chamber. Samples were oxidized with H_2O_2 and analyzed as nitrate; they have been recalculated as NO_2 (e.g., by Gray, 1959). Hasegawa entered the chamber on three occasions, and his observations on his reactions are summarized below:

NO_2, ppm	Time, min	Effects
62	60	Laryngeal irritation
25-100	120	Marked mucosal irritation
158	10	Coughing, mucosal irritation, lacrimation, headache, nausea, vomiting. Symptoms subsided after 7 hours, and no subsequent effect was observed

(Comment: The exposure probably included NO and HNO_3.)

B. Animal

• Hine CH, Meyers FH, Wright RW: Pulmonary changes in animals exposed to nitrogen dioxide, effects of acute exposures. Toxicol Appl Pharmacol 16:201-213, 1970

Review:

Animals used and their average weights were Swiss-Webster mice, male (20 g); Long-Evans rats, male (200 g); and guinea pigs (3 kg), rabbits (2.5 kg), and dogs (10 kg) of either sex and no particular strain. They were observed for 7 days before use and accepted if judged free from respiratory infection.

Exposure chambers of 200-, 1,000-, and 10,000-liter capacity were used. Air mixing was by baffle plates and fans. Air changes were 5 to 20 per hour, and total animal volume did not exceed 1/50 of the chamber volume.

Animals were exposed individually in multicompartmented cages and housed individually after exposure. Controls were held in laboratory air.

Commercial NO_2 (Matheson) was metered by capillary/manometer or by constant-delivery reciprocating syringe; the apparatus was held in a ventilated hood at 30° C to prevent condensation. Chamber air was filtered room air. NO_2 concentrations were measured by the method of Saltzman[79] or of Patty and Petty.[74]

Animal mortality was recorded hourly postexposure up to 8 hours, then daily. Selected survivors and controls were sacrificed at approximately 30, 60, 90, 180, and 360 days.

Potential cofactors studied included:

Cold	36 rats were held for 72 hours at 4° C; 32 survivors were exposed to NO_2.
Heat	20 rats were held for 24 hours to 100° F and then exposed to NO_2.
Exercise	40 rats swam to near exhaustion; 18 survivors were exercised again and then exposed to NO_2 and 18 were exposed to NO_2 and then exercised again.
Previous exposure	rats were exposed to 75 ppm NO_2 for 1 hour, a level causing only slight mortality, and then, 15 or 30 days later, at levels causing fractional mortality.

Lungs of animals dying or sacrificed were fixed in formalin and given to the pathologist without identification of experimental treatment. Sections were stained with hematoxylin and eosin or with Van Glissen and periodic acid-Schiff stains for connective tissue and hyaline membrane.

Exposure to 5, 10, or 20 ppm NO_2 for up to 1440 minutes caused no deaths in 40 rats, 52 mice, 18 guinea pigs, 12 rabbits, and 4 dogs. The only visible response was slight signs of irritation; there was no gross pathology and only questionable microscopic evidence of congestion and interstitial inflammation. These animals are not included in the table of results.

At or above 40 ppm NO_2, eye irritation, lacrimation, red conjunctivae, and respiratory distress were seen. Intense exposures occasionally caused sudden death, attributed by the authors to laryngeal spasm. Most deaths occurred within 2 to 8 hours postexposure and nearly all in 24 hours; some animals died later from pneumonitis and secondary

bacterial infection. Animals with slight pulmonary edema (detected by auscultation) and no pneumonitis looked "reasonably normal" after 96 hours.

Acute deaths were characterized by marked bronchiolitis, desquamated bronchial epithelium, infiltration by polymorphonuclear cells, and edema fluid in the alveoli. Interstitial fibrosis occurred in about one-third of the survivors at 30 days and persisted in some up to 6 months.

The results (Table A-1) showed that the effects of high concentration and brief exposure were much more severe than those of equivalent (equal Ct) low concentration and long exposure. Guinea pigs were more sensitive than rats or mice, which were more sensitive than rabbits or dogs.

Some stresses increased mortality: cold or exertion (postexposure only). Heat showed no effect. Previous exposure decreased mortality (at lower C or Ct only).

The authors concluded that there are four kinds of responses: laryngeal spasm and acute asphyxia; acute pulmonary edema; bronchiolitis and pneumonia; and recovery with permanent residua in the lungs.

Analysis:

The stated weight of guinea pigs is obviously in error and presumably should be 0.3 kg.

It is not stated if the pre-exposure observation of freedom from respiratory infection was checked by sacrifice and examination of samples; however, all postmortem signs were evaluated in comparison with unexposed controls.

The described method of exposure does not include details such as equilibration of concentration pre-exposure, method of making brief (5 minutes) exposures, or frequency of monitoring during long exposures (up to 1 day). It is unclear why chamber air was filtered when controls were exposed to laboratory air. The report does not state to what extent the generally accepted Saltzman method of analysis was used or that of Patty and Petty; the latter method uses alpha-naphthylamine (a volatile carcinogen) and has therefore not been employed or subjected to comparative evaluation in recent years.

Our own statistical analysis of the mortality data shows no more than expected variance; this suggests that the data form a consistent base for dose-response analysis.

TABLE A-1

Summary of Acute Toxicity of Nitrogen Dioxide For Five Species

Concentration, ppm	Time, min	Ct x 10^{-3}	Mortality				
			Rat	Mouse	Guinea pig	Rabbit	Dog
40	60	2.4	0/6	0/13	0/6	—	—
	480	19.2	0/6	—	2/6	—	0/3
	1440	57.6	0/10	—	—	—	—
45	180	8.1	—	0/10	—	—	—
50	60	3	0/17	0/5	1/6	0/4	0/1
	120	6	0/12	0/5	1/6	—	0/2
	240	12	0/12	0/5	—	—	0/2
	480	24	0/12	0/5	4/6	0/4	0/2
	1440	72	3/10	5/10	—	0/4	—
65	120	7.8	0/9	—	—	—	—
75	60	4.5	3/31	1/6	1/4	1/8	0/2
	120	9.0	1/12	2.6	3/4	0/6	0/2
	240	18	7/12	5/6	2/4	2/8	1/3
	480	36	12/12	6/6	4/4	6/8	1/4
85	60	5.1	6/12	—	—	—	—
	240	20.4	5/10	—	—	—	—
100	30	3	0/5	2/10	1/2	1/3	0/2
	60	6	3/5	8/10	2/2	0/4	—
	120	15	8/8	13/14	3/4	2/4	1/3
	150	15	9/11	—	—	—	—
	180	18	10/10	—	—	—	—
	240	24	29/29	10/10	—	3/4	2/2
	480	18	—	10/10	—	—	—
125	5	0.6	—	0/6	—	—	—
	30	3.7	—	4/6	—	—	—
	60	7.5	—	6/6	—	—	—
	240	15.0	—	6/6	—	—	—
150	5	0.7	—	—	—	0/2	—
	30	4.5	2/10	—	3/4	—	—
	60	9.0	10/13	—	—	1/6	2/3
	120	18.0	10/12	—	3/3	—	—
	240	36.0	4/4	—	—	3/4	—
200	5	1	6/12	4/6	2/2	0/2	—
	10	2	8/12	6/6	—	1/2	—
	20	4	5/5	6/6	—	2/4	2/2
	30	6	4/4	—	—	—	—
240	20	4.8	4/4	—	—	—	—
250	5	1.2	2/4	—	—	—	—
	10	2.5	2/4	—	—	—	—

• Carson TR, Rosenholtz MS, Wilinski FT, Weeks MH: The responses of animals inhaling nitrogen dioxide for single, short-term exposures. Am Ind Hyg Assoc J 23:457-462, 1962

Review:

The authors sought to determine exposures causing little or no effect in animals, as a basis for estimating safe exposures of man.

Commercial NO_2 (Matheson) was metered into a 400-liter dynamic exposure chamber through an expansion flask and manometer in a 31° C constant temperature box. Chamber air was sampled by evacuated bottles, before and once during 5-minute exposures and two or three times during longer exposures. Samples were analyzed by Salzman's method.[79] The authors state that there was very little variation between samples.

Animals used were young male rats, 100 to 120 g. (Rabbits and dogs were also exposed, but results are not reviewed here because they were not used in the present study.) Rats exposed at high levels (see below) were examined for gross pathologic changes only; after low-level exposure, lungs were also fixed in formalin and examined microscopically after staining with hematoxylin and eosin.

The study first determined LC_{50} values for 5-, 15-, 30-, and 60-minute exposure of rats to NO_2 (Table A-2). Rats were exposed in groups of 10. LC_{50} values were calculated by the method of Bliss as described by Finney.

TABLE A-2

Lethality of NO_2 in Rats

Exposure time, min	LC_{50}, ppm	95 % confidence limits, ppm	LCt_{50}, ppm min x 10^{-3}
5	416	376-461	2.1
15	201	191-212	3.0
30	162	152-169	4.9
60	115	113-117	6.9

The toxic signs shown by the exposed rats included severe respiratory distress, eye irritation, and death. Times of death varied from 30 minutes to 3 days after exposure, but those animals surviving 3 days after exposure appeared to recover from the respiratory distress and eye irritation.

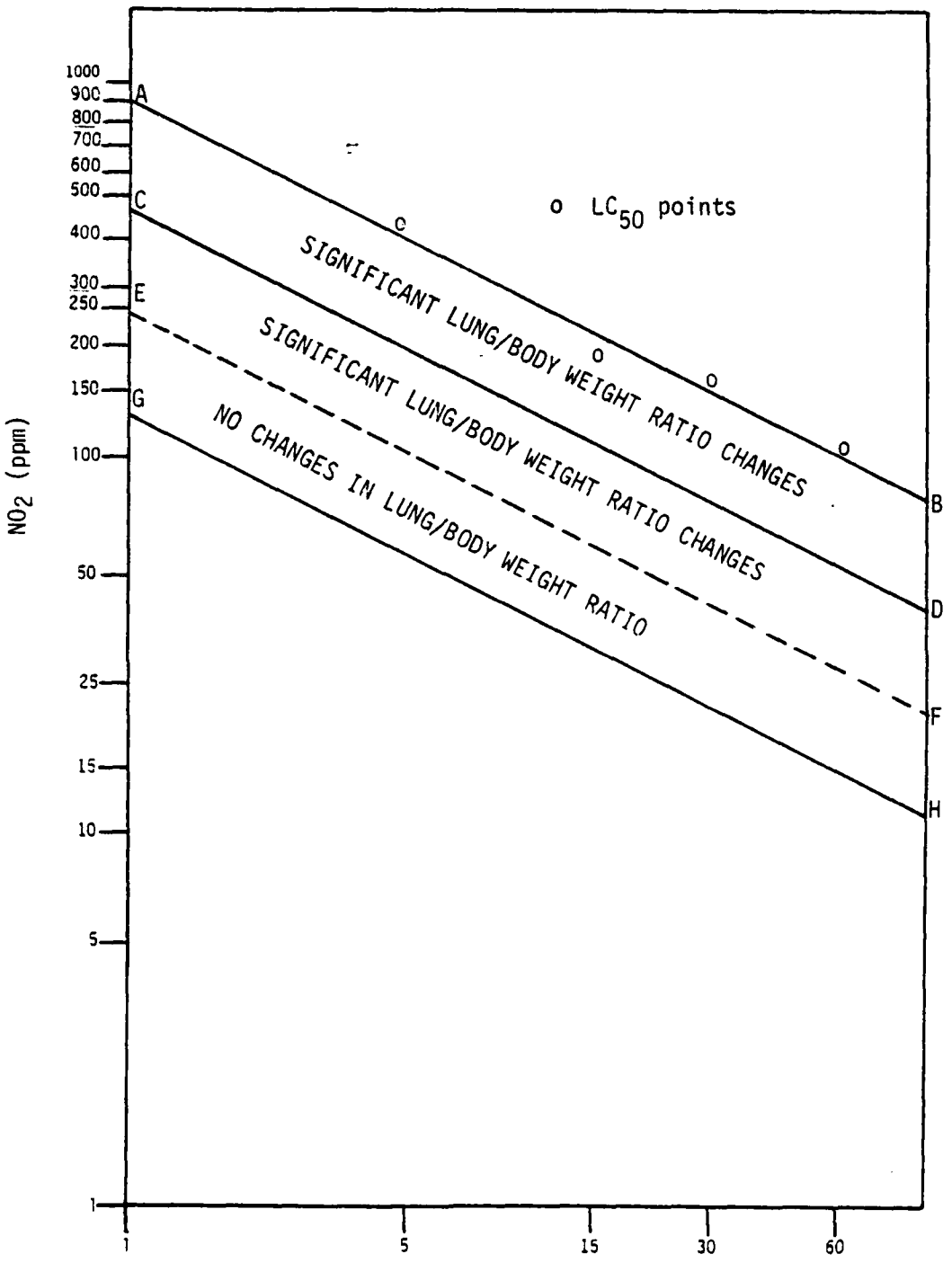

Figure A-1. Response of rats to NO_2.
Adapted from Carson et al.[12]

Rats were then exposed for 5, 15, and 60 minutes at concentrations approximating 50 percent, 25 percent, and 15 percent of the corresponding LC_{50} values. At 50 percent, rats showed severe respiratory distress and eye irritation for about 2 days, but none of them died. Microscopic studies showed pulmonary edema during the 48 hours after exposure; no change was seen in other organs. At 25 percent of LC_{50} for 5 and 15 minutes, rats showed some respiratory distress, but recovered within an hour; at 25 percent of LC_{50} for 60 minutes, they exhibited only mild nasal irritation. Pulmonary edema, but no other histologic sign was seen in some rats at 24 and 48 hours. At 15 percent of LC_{50}, rats showed no pathologic changes in comparison with controls.

Lung/body weight ratios were used as an index of response. In Figure A-1, line AB represented LC_{50} values and line CD indicated one-half of LC_{50}; the area ABDC, therefore, indicated severe toxic effects. Line EF represented one-quarter of LC_{50}, and the area CDFE indicated significant to borderline lung/body weight changes and moderate to mild respiratory and ocular irritation. No toxic effects were observed at the level of line GH. The threshold of effect line, EF, corresponded to 104 ppm NO_2 for 5 minutes, 65 ppm for 15 minutes, and 28 ppm for 60 minutes.

Analysis:

Details of the control of exposure are scanty, with no reference to method of mixing NO_2 and air, flow rate through the dynamic chamber, or method of exposing animals. If, however, the authors' statement is accepted, chamber concentrations were acceptably uniform and constant during all exposures.

The authors state that histopathologic examination was complicated by the presence of chronic murine pneumonia, which progressed as postexposure time increased. However, the lung/body weight data showed a systematic relation to intensity of exposure and are, therefore, probably acceptable as an index of pulmonary edema.

The LC_{50} values fit a log-log line well and conform to the model $Ct^a = k$.

• DiPasquale LC, Davis HV: The acute toxicity of brief exposures to hydrogen fluoride, hydrogen chloride, nitrogen dioxide, and hydrogen cyanide singly and in combination with carbon monoxide. Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio, AMRL-TR-71-120, Paper No. 20, 1970; NTIS AD-751 442, 1971. (The work is also reported in Higgins EA, Fiorca V, Thomas AA, Davis HV: The acute toxicity of brief exposures to HF, HCl, NO_2 and HCN singly and in combination with CO. Report No. FAA-AM-71-41, Nov. 1971, 8 pp; NTIS AD-735 160.)

Review:

This study was performed to determine if CO affected response to NO_2 and other toxic gases in brief intense exposures, in connection with fire hazards in aircraft.

Male Wistar rats, 250 to 275 g, were exposed in groups of 10, and male ICR mice, 30 to 35 g, in groups of 15. A dynamic exposure chamber was used with sliding wire cage drawers with solid gasketed ends for rapid insertion and withdrawal. NO_2 was supplied from a cylinder in a waterbath. Air samples were absorbed in Saltzman reagent[79] and assayed in a Technicon Autoanalyzer calibrated by both vapor bag and permeation tube. CO was metered in to give approximately 25 percent carboxyhemoglobin (COHb) in animals immediately after exposure; in preliminary 5-minute exposures, it was found that the necessary concentrations were 2100 ppm for rats and 1500 ppm for mice.

When the desired concentration was achieved, the cage was pushed in and clamped; after 5 minutes it was withdrawn. The concentration was adjusted to another level and the procedure was repeated. Animals were observed for 7 days, and mortality data were analyzed by the method of Litchfield and Wilcoxon.[57]

Results are shown in Tables A-3 and A-4.

The only response observed during exposure was severe respiratory distress. Most deaths occurred within 24 hours and the rest within 48 hours; exposure to CO did not alter the time to death. The mortalities in NO_2 and NO_2 + CO were not significantly different, but the species response was significantly different. Gross pathology indicated that death resulted from pulmonary edema, with a few animals showing pulmonary hemorrhage.

The authors conclude that CO exposure giving 25 percent COHb does not increase the hazard from NO_2 exposure since there is no enhanced mortality or decreased time of survival.

Analysis:

The experimental technique is generally sound but some details that would assist evaluation are not given, e.g., the flow rate (or air changes per hour) in the chamber, the method of mixing gases with the air flow, sampling to verify uniform exposure, and method of analysis for CO. The last point would be well covered if blood samples were taken after exposures, as in the preliminary CO exposures, but there is no indication that this was done.

The statistical method is a rapid graphic technique that accommodates zero and 100 percent mortality as well as fractional responses. It has been widely used, but less so since computers became generally available.

Some very early deaths (during or very soon after exposure) from respiratory spasm and asphyxia would have been expected at the highest concentrations, perhaps they occurred but were attributed to pulmonary edema.

TABLE A-3

7-Day Mortality Response of Rats Exposed For 5 Minutes to NO_2 Singly and in Combination With CO (25% Carboxyhemoglobin)

NO_2 Concentration, ppm	% Deaths NO_2	% Deaths NO_2 + CO
260	10	
270		0
550	40	
580		0
590	30	
750		20
840	30	
850	30	
1000		40
1200	50	
1250	90	
1380	100	
1060		80
LC_{50}	831 ppm	1,140 ppm
95% Confidence Limits	556-1,240 ppm	720-1,707 ppm

TABLE A-4

7-Day Mortality Resopnse of Mice Exposed For 5 Minutes to NO_2 Singly and in Combination With CO (25% Carboxyhemoglogin)

NO_2 Concentration (ppm)	% Deaths NO_2	% Deaths NO_2 + CO
260	7	
550	27	
580		0
590	0	
840	7	
850	0	
950		7
1,200	40	
1,250	13	
1,380	47	
1,500		53
1,900	20	
2,280		74
2,560	67	
2,950	74	
2,980	100	
3,280		93
LC_{50}	1,880 ppm	1,044 ppm
95% Confidence Limits	1,345-2,626 ppm	1,203-2,247 ppm

• Gray EL: Oxides of nitrogen: their occurrence, toxicity, hazard. A brief review. AMA Arch Ind Health 19:479-486, 1959

Review:

Gray reviewed the toxicity of NO_x and compared some of his own findings with those of other researchers. He noted that NO and NO_2 were so closely related that the discussion of one necessarily involved the other and that chemical analysis can be at fault.

He gave his own data for male rats exposed to NO_2:

Time, min	LC_{50}, ppm	LCt_{50}, ppm.min
2	1,445	2,890
5	833	4,165
30	174	5,220
60	168	10,080
240	88	21,120

He quoted conflicting results for animal and human response in work done by other researchers, and attributed this to defective chemical analyses of NO and NO_2.

The results of Lehmann and Hasegawa's[56] study were presented with concentrations recalculated as NO_2 ppm:

NO_x as NO_2, ppm	Effects
37	Tolerated for a few hours
74	Borne only for 1/2 hour
117-154	Directly dangerous
234-388	Danger increased rapidly
776	Kills animals quickly

Gray noted that the clinical course in fatal poisoning by NO_2 is similar to that in phosgene poisoning: 82 percent of 105 persons exposed to phosgene and 83 percent of 63 exposed to NO_2 died within 48 hours.

Gray found no pathologic signs except in the lung, where edema, emphysema, and pneumonitis were seen. He observed the presence of methemoglobin after NO exposure but not after NO_2 exposure. He concluded that the main cause of conflicting evidence in reports of NO_x poisoning was the unknown or inadequately measured proportion of NO_2, the more toxic component. He considered red fuming nitric acid to be slightly more toxic than its NO_2 content would indicate, most likely because of the irritancy of the acid.

Analysis:

Being a review article, the paper does not include experimental details. However, it is useful as a source of data that should be acceptable because of the details in earlier reports from the same laboratory. (It is included here, rather than with the other review articles, because it gives original data and is of limited scope.)

Gray recalculates Lehmann and Hasegawa's NO_x concentrations as NO_2, but does not clearly justify this procedure. However, their gas was formed by the reaction of nitric acid and copper and would have consisted mainly of NO_2 if concentrated acid was used.

The observed similarity in clinical course between NO_2 and phosgene is supported by observations with other irritant and tissue-destroying gases such as chlorine.

Absence of pathologic signs except in the lung is an observation made by other investigators in animals after single acute exposure.

- Stephens RJ, Freeman G, Evans MJ: Early response of lungs to low levels of nitrogen dioxide. Arch Environ Health 24:160-179, 1972

- Evans MJ, Stephens RJ, Cabral LJ, Freeman G: Cell renewal in the lungs of rats exposed to low levels of NO_2. Arch Environ Health 24:180-188, 1972

These two papers were published concurrently and are reviewed together here.

In the first paper, intraperitoneal injection of tritiated thymidine and autoradiography was used to follow changes in lung cell proliferation after exposure of rats to NO_2. The labeling index showed an increase in the terminal bronchioles and alveoli after 2 to 3 days of exposure, more so at 17 ppm than at 2 ppm, but then returned to normal during further exposure.

The second paper demonstrated "the subtle ways in which tissue recognizes the injurious agent (NO_2) and appears to defend itself." In the first 3 days at 17 ppm NO_2, changes seen in the terminal bronchioles and alveoli included loss of cilia, thickened tissues, early (4 to 24 hours) sloughing of type 1 cells from alveolar epithelium and

subsequent (24 to 48 hours) replacement by cells that were more resistant and that thickened the air-blood barrier. At 2 ppm, changes included loss of cilia, hypertrophy, and hyperplasia with apparent return to normal after 21 days of exposure.

• Dowell AR, Kilburn KH, Pratt PC: Short-term exposure to nitrogen dioxide. Effects on pulmonary ultrastructure, compliance, and the surfactant system. Arch Intern Med 128:74-80, 1971

A volume respirator was used to expose 11 anesthetized beagles via tracheostoma to NO_2 (3 to 16 ppm); controls received air only. NO_2 was analyzed by the Saltzman method.[79] At 7 ppm and up, acute pulmonary edema was observed within 1 hour; at lower concentrations, membrane damage without edema. Cardiac output, heart rate, and systemic and pulmonary arterial pressures were all depressed. The arterial partial pressure of O_2 was depressed, the partial pressure of CO_2 was raised, and pH was lowered out of proportion to the increase in CO_2. Impaired surfactant activity and lung compliance were observed, but only with atelectasis (collapse of lung tissue) or intraalveolar edema. Below 5 ppm, "only subtle ultrastructural changes" were seen in cell membranes and mitochondria. The authors concluded that the data supported the current occupational threshold limit value of 5 ppm, at least for short-term exposure.

• Guidotti TL, Liebow AA: Toxic inhalation of nitrogen dioxide in canines. Int Conf on Photochemical Oxidant Pollution and Its Control, U.S. EPA, Raleigh, N.C., 1976, pp. 545-553; NTIS PB-264 232, 1977

Five anesthetized dogs were exposed to 37.2 ppm NO_2 for 4 hours by a novel intubation technique that exposed the left lung and kept the right lung as control. Observations were made throughout. The animals were then killed immediately and lungs were examined by light microscopy and electron microscopy.

O_2 uptake fell to 65 percent in 30 minutes. The causes were believed to be redistribution of pulmonary perfusion and perhaps regional change in airway resistance. Morphometry indicated incipient interstitial edema and early endothelial involvement. The authors observed that the study of NO_2 "is assembling a picture of generalized diffuse alveolar damage."

• Wagner WD, Duncan BR, Wright PG, Stokinger HE: Experimental study of threshold limit of NO_2. Arch Environ Health 10:455-466, 1965

Dogs, rabbits, guinea pigs, rats, hamsters, and mice were exposed to 1, 5 ,and 25 ppm NO_2, 6 hours/day, 5 days/week, with sacrifice at 3, 6, 9, 12, and 18 months. Cylinder NO_2 was used (100 percent, or 1 percent in N_2), and analysis by the Saltzman method[79] showed good control of exposures at intended levels. Exposure was in dynamic chambers; animals

were exposed singly or (guinea pigs, mice) in groups. Three weeks of conditioning "exposure" (no NO_2) preceded the experiment. All exposed groups were matched by unexposed controls.

Results were largely negative: no significant difference in weight gain, no hematologic change (Hb, hematocrit, WBC), no significant change in basic alkaline phosphatase or Mg-activated phosphatase in blood sera, no clear change in respiratory function (except an indication in rabbits exposed to 25 ppm), no conclusive evidence in gross pathology or histopathology.

There was an indication of tumor acceleration in a spontaneous tumor strain of mice (CAF_1/Jax) at 1 yr, not thereafter. The authors did not claim a definite effect but recommended that the 5 ppm occupational exposure standard should be redesignated as a ceiling rather than an average.

There was clear evidence of induced tolerance in mice and rats exposed at 5 ppm and 25 ppm and challenged in one 5-hour exposure of 60-70 ppm; considerable protection resulted.

	Exposure, weeks		24-hour mortality after challenge at 60-71 ppm for 5 hr	
	5 ppm NO_2	25 ppm NO_2	Pre-exposed	Controls, not Pre-exposed
Mice	7	--	5/14	9/14
Mice	--	7	0/21	6/21
Rats	56	6	0/6	4/6
Rats	56	6	0/6	4/6

o Kleinerman J: Effects of nitrogen dioxide on elastin and collagen contents of lung. Arch Environ Health 34:228-232, 1979

Hamsters were exposed to 30 ppm NO_2 22 hours/day for 3 weeks. There was a general loss in body weight and increase in dry lung weight compared with controls, reversed after exposure. Lung collagen was significantly decreased on the 4th day and recovered during exposure; lung elastin was decreased on the 10th day and did not recover until exposure was discontinued.

o Ehrman RA, Treshow M, Lytle IM: The hematology of mice exposed to nitrogen dioxide. Am Ind Hyg Assoc J 33:751-755, 1972

Swiss Webster mice were exposed to NO_2 for 1 hour. At 10 ppm, females showed decreased hemoglobin (Hb) and erythrocytes, and increased bilirubin and methemoglobin (MetHb); males showed only the increased bilirubin and MetHb.

The authors speculated that the apparent hemolytic anemia was caused by the ions (nitrite, nitrate) formed from NO_2 by reaction with water, and suggested a mechanism for their interference with cellular respiration.

2. Exposure to NO_2: Animal Infectivity Model

• Ehrlich R: Effect of nitrogen dioxide on resistance to respiratory infection. Bacteriol Rev 30:604-614, 1966

Review:

This paper presents new data as well as reviews earlier work on NO_2 with emphasis on decreased resistance to respiratory infection. It deals first with acute exposures up to 2 hours and then chronic exposures up to 3 months.

Swiss albino mice were exposed to NO_2 for 2 hours before exposure to Klebsiella pneumoniae for 10 minutes and then observed for 14 days. The NO_2 was metered from a cylinder of 1 percent (10,000 ppm) NO_2 in air. The bacterial aerosol particles were 1 to 5 microns in diameter. Control mice were exposed to NO_2 only or K. pneumoniae only. Enhanced mortality from bacterial pneumonia was observed in mice exposed to 3.5 ppm NO_2 and higher concentrations but not at 2.5 ppm or below. The survival time of enhanced-mortality mice was less than that of control mice dying after exposure to K. pneumoniae only. There were no deaths from exposure to NO_2 only.

Similar results were obtained with mice exposed first to K. pneumoniae and then to NO_2 within 1 hour. When exposure to NO_2 (at 25 ppm) was delayed for 6 or 24 hours, there was no significant difference in mortality enhancement. However, when similar delays were introduced in exposure of mice to NO_2 and then to K. pneumoniae, the enhancement was less at 6 hours and absent at 27 hours; this was observed at NO_2 concentrations for 5 to 25 ppm. The author comments that this evidence suggests that NO_2 causes reversible damage to pulmonary defense mechanisms.

The resistance of Swiss albino mice and 4 inbred strains was compared. BDF_1 and $C_{57}BL/c$, were more resistant to K. pneumoniae infection than SA, BALB/c and LAF_1. BDF_1 and LAF_1 were more resistant in exposures to NO_2 before bacterial challenge, and $C_{57}BL/c$ was more resistant in exposures to NO_2 after bacterial challenge. The author comments that strain differences relate more to infection than to response to NO_2.

Hamsters are highly resistant to K. pneumoniae and showed no mortality enhancement up to 25 ppm NO_2; at 35 ppm and above, enhancement was highly significant. Squirrel monkeys showed mortality enhancement from 2 hours exposure to 40 ppm NO_2 before bacterial challenge.

The effect of NO_2 on viable counts of retained bacteria was studied in Swiss albino mice and golden hamsters. Animals were exposed for 2

hours to NO_2 at 5 to 50 ppm and then to K. pneumoniae for 10 minutes. They were killed at 0, 1, 3, 5, 6, 7, or 8 hours after bacterial exposure, and the lungs were homogenized and cultured quantitatively. Control animals were exposed to K. pneumoniae only. Bacterial counts were expressed as percentages of the zero-time counts. In mice, counts were approximately 70 percent to 80 percent at 1 hour. The control mice showed a further drop to approximately 30 percent to 40 percent at 5 to 6 hours; and the counts then increased rapidly. In mice exposed to NO_2, this further drop was much reduced or did not occur, and the time for counts to return to 100 percent was shorter at higher NO_2 concentrations. Similar results were seen in hamsters.

The initial recovery of bacteria was variable and always less in NO_2-exposed animals. The author comments that increased mortality might be explained by damage to ciliary and phagocytic activity.

The author notes that NO_2 in air pollution may reach 3.5 ppm and that diurnal variations are wide. He describes chronic exposure of Swiss albino mice to 0.5 ppm NO_2 followed by bacterial challenge. Control mice breathed clean air before challenge. There was a significant increase in mortality among NO_2-exposed mice after 3 months.

Continuous exposure for 30 days to 0.5 ppm NO_2 was compared with intermittent exposure for 30 days at 6 hours/day, 5 days/week. The intermittent exposure gave significantly greater enhancement of mortality. In continuous exposure of Swiss albino mice for 90 days at 1.5 ppm, significant enhancement was seen at 8 hours and thereafter.

The author comments that almost all response to NO_2 in man and other animals is confined to the respiratory tract, in lethal exposure or sublethal exposure with pathologic changes. Enhancement of mortality is a more sensitive indicator: 2 hours at 3.5 ppm NO_2 showed a significant but reversible effect, and response was observed after intermittent exposure to 0.5 ppm NO_2 for 30 days or continuous exposure for 3 months. The author suggests that the causative mechanism may be that NO_2 "permits better colonization" and that the work suggests a possible effect of air pollution on human resistance to respiratory infection.

Analysis:

The paper does not give many experimental details, which is appropriate in a review article citing papers in which details may be found. Reference to these papers provides ample evidence that the data should be accepted as valid.

• Ehrlich R, Henry MC: Chronic toxicity of nitrogen dioxide. I. Effect on resistance to bacterial pneumonia. Arch Environ Health 17:860-865, 1968

Review:

Mice were exposed to 0.5 ppm NO_2 for 6, 18, or 24 hours/day. Groups withdrawn at intervals were exposed to airborne K. pneumoniae. Mortality from bacterial pneumonia was compared with that of control mice held in clean air before bacterial challenge.

Exposed and control mice were held in two similar walk-in chambers. Room air was passed through a fiberglass filter and then through activated charcoal into each chamber to give approximately 20 changes per hour. NO_2 (99.5 percent minimum) from a cylinder was passed continuously through a heated tube into a mixing vessel where it was diluted with air and then passed into the exposure chamber. Grab samples were taken from different sections of the chamber and analyzed for NO_2 by Saltzman's method.[79] Continuous monitoring with a Mast gas analyzer gave results that agreed with the chemical method. The control chamber was also monitored.

Female Swiss albino mice (initial weight approximately 21 g) were held in four groups of 30 cages each as follows:

	7 days/week routine	
Group	NO_2 chamber	Control Chamber
24 hours	24 hours	--
18 hours	3 pm-9 am	9 am-3pm
6 hours	9 am-3 pm	3 pm-9 am
Control	--	24 hours

Mice were withdrawn at 1, 3, 6, and 12 months for bacterial challenge; they were replaced by other mice to maintain the chamber population. At least 30 mice per group were exposed to K. pneumoniae and observed for 14 days.

In addition, groups of 4 to 8 mice were killed immediately after bacterial challenge, the lungs were removed and homogenized in sterile distilled water, and the homogenate was cultured on blood agar. Similar groups of exposed and control mice were killed and assayed hourly up to 8 hours and at 24 hours after challenge. The clearance rate of viable K. pneumoniae was determined by expressing the remaining bacterial counts as percentages of the initial count.

Results of a previously reported study of continuous exposure to 0.5 ppm NO_2 for 7 days to 9 months were aggregated with the 24-hour group in this study for analysis. In continuous exposure, a statistically significant increase in mortality was observed at 3 months and thereafter; earlier observations showed a nonsignificant increase. In intermittent exposure, the 18-hour group showed a nonsignificant increase at 3 months and the 6-hour and 18-hour groups showed a significant increase at 6 months, but both groups showed lower mortality than their control groups at 12 months.

Decreased bacterial clearance rates were seen in the 24-hour group after 6 months, the 18-hour group after 9 months, and the 6-hour group only at 24 months.

The authors discuss two hypothetical mechanisms for enhancing mortality: (1) stimulation of bacterial growth by NO_2, directly or by release of a nutritional factor from the lung, and (2) impairment of defense against infection by mucociliary clearance and phagocytosis. They consider impairment of defenses, especially of macrophage activity, the more likely.

Analysis:

The analytical methods for NO_2 are acceptable, but no data are given to show spatial and temporal variations from the nominal concentration of 0.5 ppm NO_2, or to substantiate the stated agreement between the Saltzman method and the Mast analyzer.

The procedures for holding in the control chamber when not exposed in the NO_2 chamber, and for replacing test mice, show careful attention to controlled uniformity of exposure conditions. However, the type of cage and number of mice per cage are not given, so one cannot judge how effectively the mice were exposed to the chamber atmosphere.

• Blair WH, Ehrlich R, Henry MC: Chronic toxicity of nitrogen dioxide: II. Effect on histopathology of lung tissue. Arch Environ Health 18:186-192, 1969

Review:

This report is on mice from the study reported by Ehrlich and Henry (1968); see the review of that article for more experimental detail.

Mice were exposed to 0.5 ppm NO_2 for 6, 18, or 24 hours/day and controls were held in clean air. Up to 4 mice from each group were killed after 1, 3, 6, 9, or 12 months of exposure. Tissues were fixed in Bouin's solution, embedded in paraffin, sectioned at 8 microns, dewaxed in xylene, stained with hematoxylin and eosin, and observed at x 100 and x 400 magnification. All the significant observations were in lung tissue; no unique pathologic signs were observed in heart, liver, kidney, or spleen.

Control mice showed moderate pneumonitis consistent with advancing age but no bronchiolar obstruction or emphysema.

Mice exposed to NO_2 showed inflamed bronchioles, surface erosion of epithelium, blockage of bronchiolar-alveolar junctions by debris, and alveolar expansion. There was an overall trend towards increased response with time but no consistent difference between intermittent and continuous exposure.

The authors found the overall lesions to be consistent with microscopic development of early focal emphysema and noted that 0.5 ppm NO_2 was the lowest level at which such effects had been seen in experimental animals.

Analysis:

See review of Ehrlich and Henry (1968) for analysis of experimental procedures in animal exposure.

The pathological procedures reported here represent standard practice.

• Henry MC, Ehrlich R, Blair WH: Effect of nitrogen dioxide on resistance of squirrel monkeys to Klebsiella pneumoniae infection. Arch Environ Health 18:580-587, 1969

Review:

Methods for NO_2 exposure and respiratory challenge with bacteria, developed in earlier work with rodents and first described by Purvis et al. (J Infect Dis 109:238-242, 1961; ibid. 113:72-76, 1963), were applied to squirrel monkeys.

Monkeys were trained to accept a respiratory mask over nose and mouth, connected to a spirometer and a mixing chamber through which air or NO_2-air passed. Exposures to NO_2 for 2 hours were preceded by 30 minutes of clean air exposure. Respiratory functions were recorded throughout the 2-hour exposure and for 15 minutes on succeeding days. NO_2 concentrations were monitored by Salzman's method[79] and continuously by a Mast analyzer.

Bacterial challenge with K. pneumoniae was made either within 1 hour after NO_2 exposure or 3 to 5 days before NO_2 exposure. The bacterial dose, calculated from aerosol concentration and respiratory minute volume, was in the range of 10^4 to 10^5 organisms.

Monkeys challenged with 10^4 K. pneumoniae showed no response; at 10^5 organisms, tidal volume decreased at about the 5th day and returned to normal in 7 to 14 days. One monkey showing more intense response was sacrificed at 24 days, and lung smears were positive for K. pneumoniae; others, sacrificed at 15 to 57 days, were negative.

Two monkeys exposed at 50 ppm NO_2 showed increased respiratory rate and decreased tidal volume, recovering in 7 days; one, rechallenged after 2 months, reacted more violently and died within 4 to 18 hours. Two monkeys exposed to K. pneumoniae soon after NO_2 exposure died in 5 to 72 hours; one monkey exposed to K. pneumoniae 24 hours after NO_2 exposure died within 72 hours.

Monkeys exposed to NO_2 only at 35 ppm showed similar respiratory changes. Two challenged with K. pneumoniae after NO_2 showed increased

respiratory minute volume at 2 days and one gave lung smears positive for K. pneumoniae.

Monkeys exposed to NO_2 only at 10 to 15 ppm showed only slight respiratory response. One of three challenged with 10^4 K. pneumoniae after 15 ppm NO_2 had lowered tidal volume 11 to 17 days postexposure; two challenged with 10^5 organisms had lowered tidal volume 4 to 12 days postexposure.

Four monkeys were challenged with 5.0 to 9.5 x 10^5 organisms and exposed to 10 ppm NO_2 3 or 5 days later; three gave lung smears positive for K. pneumoniae at 19 to 46 days and one was negative at 51 days.

Histopathology of monkeys exposed at 10 ppm NO_2 showed expanded alveoli and breaks in alveolar septa; alveolar expansion was more marked after exposure at 15 ppm NO_2 and there were signs of pathologic changes in kidney and liver. Exposure at 35 or 50 ppm NO_2 caused marked lung damage, with areas of collapse, extensive edema, erosion of bronchiolar epithelium, and absence of bronchiolar cilia. Some pathologic changes were seen in heart, kidney, and liver. The overall picture was of progressive alveolar expansion with increasing exposure to NO_2, accompanied by some other organ damage. The authors summarized the consequences of NO_2 exposure in conjunction with infectious challenge as fatalities at 50 ppm NO_2 and delayed lung clearance of bacteria at 35 ppm NO_2 and less.

Analysis:

The experimental methods had been developed and established in extensive previous studies and do not call for comment.

Despite the small number of subjects, a clear picture emerges of the influence of NO_2 on establishment of lung infection and, in more intense exposure, a fatal outcome. Evidence here and elsewhere suggests impaired pulmonary defenses as the cause but does not exclude factors positively enhancing infection.

The observation of pathologic changes in organs other than the lung is unusual, especially after acute exposure.

• Ehrlich R, Findlay JC, Fenters JD, Gardner DE: Health effects of short-term exposures to NO_2-O_3 mixtures. International Conference on Photochemical Oxidant Pollution and Its Control. U.S. EPA, Raleigh, N.C., 1976, pp. 565-574; NTIS PB-264-232, 1977.

Review:

The following review covers enhancement of respiratory infection by exposure to NO_2; the study also included exposure to O_3 and to NO_2-O_3 mixtures.

Female CF-1 and CD_2F_1 mice, 6 to 10 weeks old, were exposed individually in separate compartments of wire cages. Cylinder NO_2 (99.5 percent purity) at 1 percent in air was diluted with dried and filtered air and passed into a 432-liter chamber at 60 liters/minute. A small blower stirred the chamber air during animal exposure. NO_2 concentration was continuously monitored by a Bendix chemiluminescent analyzer. Control animals were similarly exposed to clean air.

Bacterial challenge was with Streptococcus pyogenes aerosol in a 400-liter chamber. Thawed inocula were grown in Todd Hewit broth for 18 hours at 37° C and adjusted to 65 percent optical density in 0.1 percent peptone water. The suspension was dispersed through a DeVilbiss continuous flow nebulizer at airflow 8 liters/minute. Mice were exposed in individual cage compartments for 10 minutes and observed for 14 days. Three additional mice were killed immediately, and the lungs were homogenized in peptone water. The homogenate was plated on blood agar and incubated for 48 hours at 37° C. Viable counts were 10 to 30 x 10^3 organisms per gram of lung.

Single exposures of 3 hours to NO_2 gave the following results:

NO_2, ppm	No. of mice	Mortality, %
0	790	27
1.5	277	31
2	269	34*
3.5	208	57*
5	88	67*

* Significant increase ($p > 0.05$)

Experiments with mixed NO_2-O_3 included 3-hour exposures to NO_2 alone:

NO_2, ppm	Excess mortality, %
0	--
1.5	-1.7
2	14.3*
3.5	28.2*
5	35.7*

* Significant increase ($p > 0.05$)

Multiple exposures to 2 ppm NO_2 gave increased mortality after 5 and 10 daily exposures, but not after 20 exposures.

Analysis:

The subject of the study was exposure to NO_2-O_3 mixtures and the authors' discussion is concerned entirely with this. The paper is included because it is a source of data on large numbers of animals exposed to NO_2 only. The techniques described are typical of good practices in this type of investigation. In particular, NO_2 concentration was monitored by a standard instrument; use of an air stirrer and wire cages with individual compartments would favor uniform dosing; and the range of viable bacterial counts is consistent with good laboratory procedures in aerosol challenge of small rodents.

• Gardner DE, Miller FJ, Blommer EJ, Coffin DL: Relationships between nitrogen dioxide concentration, time, and level of effect using an animal infectivity model. Int Conf on Photochemical Oxidant Pollution and Its Control, U.S. EPA, Raleigh, N.C., 1976, pp. 513-525; NTIS PB-264-232, 1977

• Gardner DE, Coffin DL, Pinigin MA, Sidorenko GI: Role of time as a factor in the toxicity of chemical compounds in intermittent and continuous exposures. Part I. Effects of continuous exposure. J Toxicol Environ Health 3:811-820, 1977

• Coffin DL, Gardner DE, Sidorenko GI, Pinigin MA: Role of time as a factor in the toxicity of chemical compounds in intermittent and continuous exposures. Part II. Effects of intermittent exposure. J Toxicol Environ Health 3:821-828, 1977

• Larsen RI, Gardner DE, Coffin DL: An air quality data analysis system for interrelating effects, standards, and needed source reductions: Part 5. NO_2 mortality in mice. J Air Pollut Control Assoc 29:133-137, 1979

Review:

The second and third references cover essentially the same work as the first, but some different details are given. The fourth reference is based on the same work, but it tabulates numbers of animals exposed and fatalities, which are not available in the earlier papers; these data have been used for statistical analysis in the present report.

Pathogen-free female Swiss albino mice, 20 to 25 g, were exposed continuously or intermittently in an 11.4-ft^3 chamber to NO_2 mixed with filtered air at a flow rate of 11.4 ft^3/minute. Animals were exposed individually, and control animals were held under similar conditions in filtered air. NO_2 was monitored continuously by chemiluminescence and thrice daily by the Saltzman method.[79] At various times, groups of 20 mice were withdrawn, combined with 20 controls (air only) and exposed together to an aerosol of Streptococcus pyogenes, generated from 5 ml of a fresh broth culture, washed thrice, and resuspended at approximately 10^6 organisms/ml.

Animals were observed for 15 days and excess mortality in exposed groups was expressed as a percentage of the survival rate in unexposed controls.

The results of continuous exposure are shown in Figure A-2. Exposures were at 0.5, 1.5, 3.5, 7, 14, and 28 ppm NO_2. All showed increased mortality, and all groups could be expressed as a straight line of percent mortality increase (vs. control) against log time. The slopes of these lines increased with NO_2 concentration. The threshold time for increased mortality was 1 hour or less at 3.5 ppm NO_2 or more, several hours at 1.5 ppm, and about 1 week at 0.5 ppm. The authors noted that a 20 percent increase in mortality would require 6150 hours at 0.5 ppm NO_2 and only 1/2 hour at 14 ppm.

Mortalities from exposure at different concentrations and times were predicted from Figure A-2 for constant values of the product Ct and tabulated as follows:

	Ct = 7 ppm.hr Mortality,		Ct = 14 ppm.hr Mortality,		Ct = 21 ppm.hr Mortality,	
NO_2, ppm	Time, hr	%	Time, hr	%	Time, hr	%
1.5	4.7	6.4	9.3	10.2	14.0	12.5
3.5	2.0	18.7	4.0	27.0	6.0	32.9
7.0	1.0	30.2	2.0	41.8	3.0	48.6
14.0	0.5	21.7	1.0	44.9	1.5	58.5
28.0	0.25	55.5	0.5	67.2	0.75	74.0

The authors noted that these results indicated that concentration had a greater influence on the observed effect than time of exposure did.

Intermittent exposures were made at 3.5 ppm NO_2 (15 daily exposures of 7 hours, 7 days per week) and 1.5 ppm (21 similar exposures). (These exposures are equivalent in cumulative dose to 1.02 ppm and 0.44 ppm continuous exposure.) Results were compared with controls continuously exposed at 3.5 and 1.5 ppm. At 3.5 ppm, continuous and intermittent exposure did not give significantly different results (although the continuous exposure gave generally higher mortality); at 1.5 ppm, continuous exposure was significantly more effective, but up to 14 days only. These results showed the effect of intermittent exposure to be similar to continuous exposure of much greater total time. The authors demonstrated this by calculating excess mortalities for similar total dosages and times. The 3.5-ppm intermittent (equals 1.02 ppm continuous) exposure gave 55 percent increased mortality at 79 hours; continuous exposure to 1.5 ppm gave only 20 percent at 79 hours. Similarly, 1.5-ppm intermittent (equals 0.44 ppm continuous) exposure gave 26 percent increased mortality at 319 hours; continuous exposure to 0.5 ppm gave only 5 percent at 319 hours.

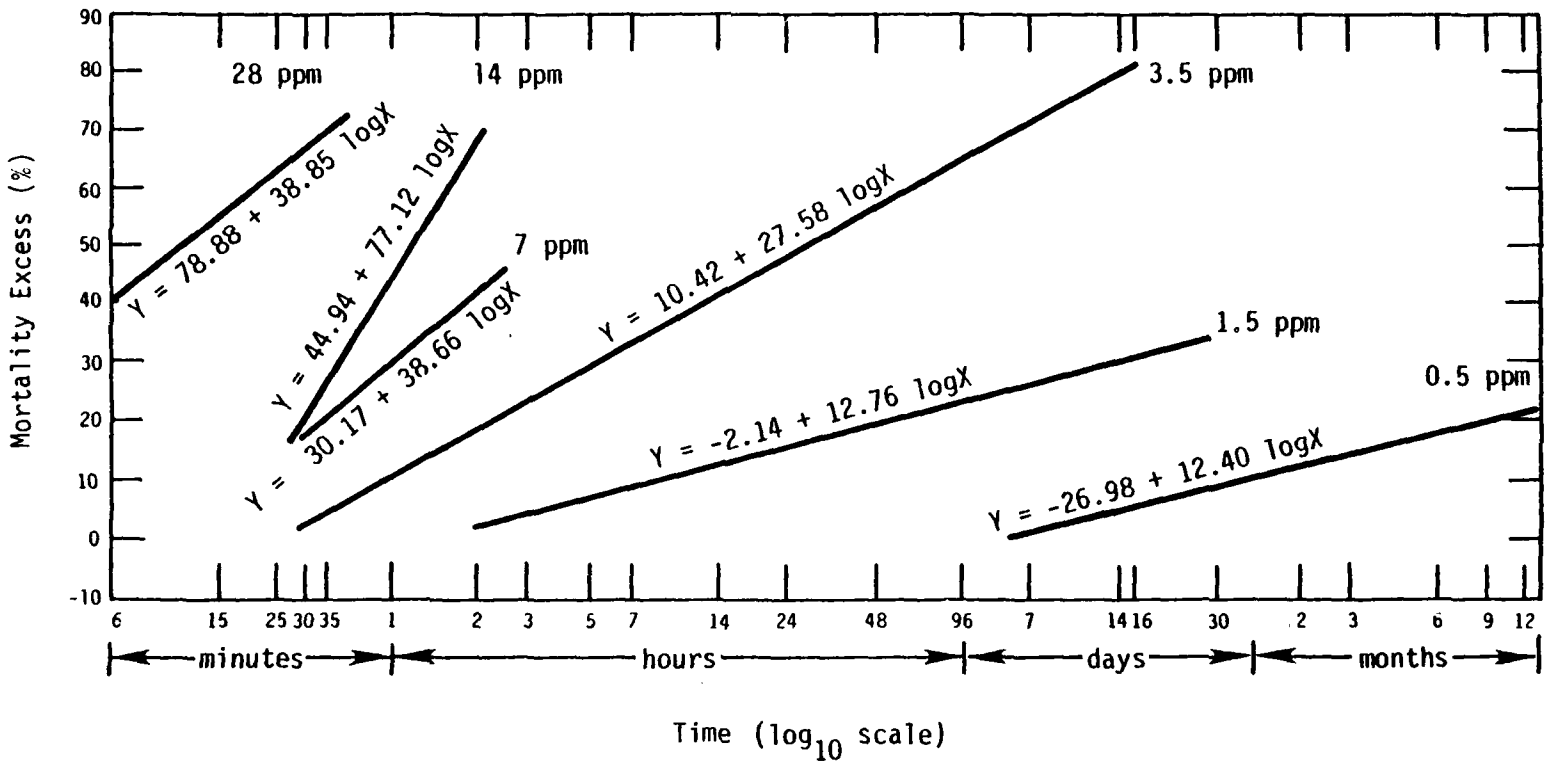

Figure A-2. Percent mortality of mice versus length of continuous exposure to various NO_2 concentrations prior to challenge with streptococci.

Adapted from Coffin et al.(1977)

Analysis:

The source of the NO_2 and the method of metering and mixing it with the chamber air supply are not given. However, the analytical procedures are acceptable and may be assumed to have shown concentrations sufficiently close to the nominal value to satisfy the investigators.

No control of aerosol challenge is reported other than the use of 5 ml at approximately 10^6 mg/ml from a fresh broth culture, washed three times.

No mention is made of any verification that all deaths were due to infection with S. pyogenes.

The calculation of equivalent exposures ignores the dominant effect of concentration, i.e., it assumes that Ct is constant for a given effect, which the authors have demonstrated is not the case.

• Goldstein E, Eagle MC, Hoeprich PD: Effects of nitrogen dioxide on pulmonary bacterial defense mechanism. Arch Environ Health 26: 202-204, 1973

Review:

Male Swiss albino mice, 25-30 g, were used. They were exposed to pure NO_2 in air for 17 hours before or 4 hours after exposure to aerosols of Staphylococcus aureus labeled with ^{32}P. NO_2 concentrations were measured at random intervals by Saltzman's method.[79] The method for quantitating intrapulmonary bacterial inactivation was as described by Green and Goldstein (J Lab Clin Med 68:669-677, 1966).

In one set of experiments, groups of 30 mice were exposed to S. aureus. Ten were killed at once and the lungs were homogenized for radioactive count and viable bacterial count; 10 mice were exposed (starting about 30 to 45 minutes after bacterial exposure) to NO_2 for 4 hours and then similarly examined; 10 were held in clean air and then used as controls. NO_2 exposure was at 1.9, 3.8, 7, 9.2, and 14.8 ppm. In another set of experiments, 17-hour exposure to NO_2 at 1, 2.3, and 6.6 ppm preceded bacterial exposure; 4 hours after this, exposed and control animals were examined as before.

In the first set, exposed to NO_2 after the bacteria, physical removal from the lung (measured by radioactivity) was not significantly affected; at 7 ppm NO_2 and above, the bactericidal activity of the lungs (measured by viable counts) was significantly depressed.

In the second set, exposed to NO_2 before the bacteria, there was no difference in inhaled dose of bacteria. This is in contrast to O_3, which had been shown to cause a profound decrease, a protective mechanism against bacterial infection not shown by NO_2. The reduction in

bactericidal activity was significant at 2.3 ppm NO_2 and very marked at 6.6 ppm. There were only minor histopathological abnormalities, even after exposure at 6.6 ppm for 17 hours; the authors concluded that it was unlikely that anatomic changes or edema contributed significantly to the impairment of bactericidal activity. Impaired macrophage activity was the most probable cause.

Analysis:

The use of controls as described would minimize potential sources of error such as inaccurate viable counts resulting from imperfect dispersal of bacteria in homogenization or from destruction of bacteria in the process.

The authors' conclusion that the experiments showed impaired bactericidal activity in the lung, and its attribution to impaired macrophage activity, are in keeping with the evidence presented and the work of others.

3. Exposure to NO

A. Human

• Von Nieding G, Wagner HM: Vergleich der Wirkung von Stickstoffdioxid und Stickstoffmonoxid auf die Lungenfunktion des Menschen. Staub-Reinhalt Luft 35:175-178, 1975

Review:

This paper summarizes earlier work of von Nieding et al. on NO_2 and compares its effects with those of NO, which had recently been reported at the third International Clean Air Congress, 1973. The experimental procedures are generally the same as those reviewed under "Exposure to NO_2," and the following review focuses on the new procedures and results for NO exposure.

Healthy (298) and bronchitic (132) volunteers were exposed to NO_2 or NO in air, inhaled via a mouthpiece with flap valve from a plastic bag. The NO-air mixture was bubbled through an alkaline solution of salicylic and sulfanilic acids to remove NO_2 and then through a filter to eliminate aerosol particles.

Alveolar (end-expiratory) O_2 was measured by mass spectrometry and arterial O_2 by electrometry. Whole body plethysmography was used to measure respiratory functions.

Results are given for exposure of bronchitics only. Some figures for statistical significance are included, but the number of subjects is not given.

Effect	Threshold Exposure for Effect
Decrease in arterial partial pressure of O_2	NO_2: 5 ppm for 15 minutes NO : above 15 ppm for 15 minutes (p 0.01)
Diffusion capacity for CO	NO_2: 5 ppm for 15 minutes NO : none at up to 39 ppm (p 0.01)
Increase in airway resistence	NO_2: 1.6 to 2.0 ppm NO : above 20 ppm

Analysis:

It is not possible to judge how successful the removal of NO_2 was from the NO-air mixture. The composition of the wash fluid and the type of bubbler are not described, nor is the time between treatment and inhalation given. There is, therefore, some doubt whether the observed decrease in arterial O_2 and increase in airway resistance were due to NO or to NO_2. However, the results are not inconsistent with the widely held view that NO is much less irritant than NO_2 and less toxic by a considerable margin.

• Clutton-Brock J: Two cases of poisoning by contamination of nitrous oxide with higher oxides of nitrogen during anesthesia. Br J Anaesth 39:388-392, 1967

Two cases are described of human poisoning by anesthetic N_2O contaminated with NO; one subject died. Signs included cyanosis and respiratory distress; loss of lung compliance was observed. Blood samples were cyanotic and had a "distinct brownish color." Methemoglobin (MetHb) was found (amount not stated). In the fatal case, the lungs were extremely edematous. The author did not detect any odor of higher nitrogen oxides when he tested the gas.

Information elsewhere shows that the cylinder N_2O probably contained 1.5 percent NO; the gas phase may have contained more, because of preferential volatilization. As breathed, in mixture with 25 percent and later 50 percent O_2, there must have been substantial oxidation to NO_2. The observations suggest a combined effect: edema from irritation by NO_2, and MetHb with cyanosis from NO.

B. Animal

Note: The first two references are to laboratory experiments undertaken in studying the accidental human poisonings with NO reported by Clutton-Brock (1967).

• Greenbaum R, Bay J, Hargreaves MD, Kain ML, Kelman GR, Nunn JF, Prys-Roberts C, Siebold K: Effect of higher oxides of nitrogen on the anaesthetized dog. Br J Anaesth 39:393-403, 1967

Twelve dogs were exposed to NO (0.5 to 2 percent) or NO_2 (0.1 to 2 percent) in O_2 (30 percent O_2 and 70 percent N_2O in one case).

Eight died at or soon after end of exposure; four were sacrificed, three after 24 to 48 hours in which "they showed no obvious signs of distress." Results are summarized below:

NO_2, %	Time, min	Result
0.1	136	Sacrificed
0.5	5-22	Sacrificed
0.5	45	Died 2 hours later
2.0	15	Died at 15 minutes
NO, %	**Time, min**	**Result**
0.5	25	Died 7 minutes later
2.0	7-50	Died at or soon after end of exposure

Death was always associated with critical reduction in arterial oxygen content (despite inhaling 98 percent O_2); this was caused by one or more of (1) formation of methemoglobin (quickly reversible by methylene blue treatment), (2) low arterial O_2, or (3) acidemia (affecting oxyhemoglobin dissociation). Low arterial oxygenation was attributed to flooding of alveoli by fluid, caused by acid hydrolysis products from NO_2 (which was formed in visible concentrations in NO exposures).

• Toothill C: The chemistry of the in vivo reaction between hemoglobin and various oxides of nitrogen. Br J Anaesth 39:405-411, 1967

A hematological study was made of dogs exposed to NO or NO_2 under anesthesia. Toothill notes that hemoglobin (Hb) has a very high affinity for NO, but this has only been demonstrated in the absence of O_2. NOHb had been reported in man, but never spectroscopically confirmed.

At 0.1 percent NO_2 in O_2 (1000 ppm), cyancsis was seen after 155 minutes, the time of the first positive methemoglobin (MetHb) reading of 4.6 percent. At 2 percent NO in O_2 (20,000 ppm), MetHb was 5.3 percent after 4 minutes and approximately 100 percent at 50 minutes. At 2 percent NO in N_2O, similar readings were made: 5.3 percent MetHb at 5 minutes. Lesser effects were seen in exposure at 0.1 percent to 0.5 percent of NO. No NOHb was detected. Eight dogs were tested and all died after exposure.

Although the observations are at NO and NO_2 concentrations beyond the scope of the present study, they do confirm the unlikelihood of NOHb formation, and the likelihood of MetHb formation, but only at low NO and NO_2 concentration.

• Oda H, Kusumoto S, Nakajima T: Nitrosyl-hemoglobin formation in the blood of animals exposed to nitric oxide. Arch Environ Health 30:453-456, 1975

A mixture of NO in air was passed through soda lime immediately before exposure of mice, rats, and rabbits in a dynamic chamber. Analysis of chamber air gave 10.6 percent NO and 0.8 percent NO_2. Animals were withdrawn at 10-minute intervals and blood was quickly analyzed for NOHb by electron spin resonance spectrometry. NOHb reached 0.13 percent of total Hb in 20 minutes and then stabilized; on removal of animals to clean air, NOHb had a half-life of 10 minutes or less. NOHb showed a dose-response relationship to NO concentration that was curvilinear, with proportionally higher NOHb at higher NO. The authors comment on the differences from in vitro tests, in which NO shows more than 1,000 times higher affinity for Hb than CO, and they speculate that the reaction is complicated in vivo by reaction of NO with O_2 and H_2O to form NO_2 and nitrite ions.

The authors report that mice exposed to 12.8 ppm NO_2 showed the same NOHb spectrum and almost the same intensity as those exposed to 10.6 ppm NO with 0.8 ppm NO_2. This apparently confirms the formation of NOHb by NO_2 alone, since the NO_2 was from a cylinder containing 10,000 ppm (1 percent) in air and would not be expected to include a significant proportion of unoxidized NO.

• Hugod C: Effect of exposure to 43 ppm nitric oxide and 3.6 ppm nitrogen dioxide on rabbit lung. Int Arch Occup Environ Health 42:159-167, 1979

Six rabbits were exposed for 6 days to 43 ppm NO and 3.6 ppm NO_2; six controls were held in clean air. NO was from a cylinder of 5 percent NO in N_2 and the NO-air mixture was passed through (1) alkaline solution of sulfanilic and salicylic acids (as in von Nieding et al.[93]) and (2) soda lime (as in Oda et al.[71]) to minimize NO_2. The gas mixture was continuously monitored by a Bendix NO, NO_2, NO_x Analyzer. After exposure, lungs were examined by eye, light microscope, and electron microscope. There was no significant change in exposed rabbits in contrast to earlier work by Hugod in which changes were seen in rabbits exposed for 14 days to 5 ppm NO. Hugod suggests various reasons for this discrepancy and notes that further work is needed.

• Azoulay E, Soler P, Blayo MC, Basset F: Nitric oxide effects on lung structure and blood oxygen affinity in rats. Bull Eur Physiopathol Resp 13:529-544, 1977

Rats were exposed for 6 weeks to 2 ppm NO; NO_2 contamination, including background, did not exceed 0.08 ppm. No methoxyhemoglobin (MetHb) was detected, nor was hemoglobin affinity for O_2 otherwise modified. No significant lung structure difference was seen in comparison with controls. The results support other indications that NO is not as toxic as NO_2 and that MetHb is not a significant factor in NO exposure at low concentrations.

4. Review Articles and Guideline Documents

• American Industrial Hygiene Association, Toxicology Committee: Emergency exposure limits. Nitrogen dioxide. Am Ind Hyg Assoc J 25:580-582, 1964

An emergency exposure limit (EEL) "can be tolerated without adversely affecting health but not necessarily without acute discomfort or other evidence of irritation or intoxication." Assumptions made by AIHA in setting an EEL include: (1) it will be a single event; (2) subjects will not be abnormally sensitive; (3) impairment of vision, judgment, and coordination may occur, but not so as to prevent self-rescue; and (4) there are no other contaminants.

The AIHA recommended the following:

Emergency exposure limits: nitrogen dioxide

5 minutes	35 ppm	175 ppm·minute
15	25	375
30	20	600
60	10	600

The concentration hazardous to life was given as 250 ppm; this "will lead to severe pulmonary effects, possibly including pulmonary edema." The threshold of odor detection for many people is about 1 to 3 ppm and some will find brief exposure at 25 ppm irritating. The AIHA's discussion is briefly summarized here:

Gray et al.[37-39] studied effects of NO_2 and red fuming nitric acid in animals; the TLV of 5 ppm adopted by the ACGIH in 1962 was based largely on Gray's findings. NO_2 is primarily a respiratory irritant causing edema and, in some survivors, bronchiolitis fibrosa obliterans. Former confusion about the different oxides involved in toxicity has been overcome by better analytical methods.

The recommended EEL's are the same as the Emergency Tolerance Limits recommended by the National Academy of Sciences in 1961.

Other evidence is in Carson et al.[12] who exposed rats to NO_2 for 5 to 60 minutes and found $Ct^a = k$. This means that for a given effect, log C vs. log t is a straight line; the constant a is less than 1.0, concentration having the greater influence. LCt_{50} values included:

60 minutes	6,900 ppm·minute
30 minutes	4,860 ppm·minute
15 minutes	3,015 ppm·minute
5 minutes	2,080 ppm·minute

Using increased lung/body weight ratio as indicator, Carson et al. drew a threshold effect line parallel to the LC_{50} line, which gave:

	Threshold	
60 minutes	20 ppm	1,200 ppm·minute
30 minutes	25 ppm	750 ppm·minute
15 minutes	35 ppm	525 ppm·minute
5 minutes	60 ppm	300 ppm·minute

Based largely on this, AIHA gave a table of expected effects in humans (modified here):

Pulmonary edema and death	5 minutes	400 ppm	2000 ppm·minute
	15 minutes	200 ppm	3000 ppm·minute
	30 minutes	150 ppm	4500 ppm·minute
	60 minutes	100 ppm	6000 ppm·minute
Pulmonary edema with possible sub-acute or chronic lesions in the lungs	5 minutes	200 ppm	1000 ppm·minute
	15 minutes	100 ppm	1500 ppm·minute
	30 minutes	75 ppm	2250 ppm·minute
	60 minutes	50 ppm	3000 ppm·minute
Respiratory irritation, chest pain	5 minutes	100 ppm	500 ppm·minute
	15 minutes	50 ppm	750 ppm·minute
	30 minutes	40 ppm	1200 ppm·minute
	60 minutes	25 ppm	1500 ppm·minute

Hine et al. (unpubl.) exposed volunteers; AIHA used the results to adapt Carson's rat data to brief human exposure. Within 1 minute at 50 ppm NO_2, 2/7 subjects had severe substernal pain. About half found brief exposure at 25 ppm unpleasant.

The immediately hazardous value of 250 ppm was extrapolated from Carson et al. and is for 1 minute exposure; this extrapolation to low t is supported by Gray et al. who obtained a linear log-log plot down to 2 minutes.

• Coffin DL, Stokinger HE: Biological Effects. Nitrogen Dioxide. In Air Pollution, 3rd Edition, Volume II, Ed. AC Stern. Academic Press, New York, pp. 264-351 1977.

This review of the biological effects of NO_2, though oriented to air pollution, is an overview of wide applicability. The authors tabulate research findings by concentration under three headings: short intermittent, long intermittent, and long continuous exposure. Results in the first are summarized briefly here for convenience. Exposure to

0.5 to 5 ppm NO_2 for 2 minutes to 4 hours elicited the following responses: structural changes in lung mast cells, collagen; peroxidation of lung lipids; reduced lung clearance of bacteria and increased mortality from respiratory infection; increased airway resistance in bronchitic humans in 2 minutes, and falling diffusion capacity in 15 minutes in healthy subjects.

The review of pathological effects includes much histopathology and some evidence of cumulative damage. A particularly interesting reference is to emphysematous changes in mice (Blair et al.[8]) in these experiments; 6 hours/day exposure was similar in effect (though less on the whole) to 18 hours/day and 24 hours/day. (Exposure was 7 days/week: Coffin and Stokinger incorrectly state 5 days/week.)

The review of physiological effects notes the observation by Vaughan et al.[69] of 90 percent NO_2 uptake by dogs in nose-trachea prep- arations. It also quotes Svorcova et al.[86] who found that 24 ppm NO_2 inhaled was "immediately" found as nitrite and nitrate ions in the blood, and in urine within 15 minutes.

Methemoglobin (MetHb) is discussed. The authors note that although in vitro exposure and ingested nitrite can be shown to form MetHb, it is difficult to demonstrate by inhalation in vivo and certainly not at "air pollution" concentrations (i.e., not more than a few ppm). This is surprising (e.g., Svorcova et al.'s finding of nitrite and nitrate in blood; see above) and, like others, the authors find the evidence confusing. They conclude that the weight of evidence suggests no more than slight and transitory formation of MetHb.

• Guidotti TL: The higher oxides of nitrogen: inhalation toxicology. Environ Res 15:443-472, 1978

Among the bioreactions of NO_x, Guidotti notes the injurious effects of HNO_3 from NO_2, free radical formation, oxidation, nitrite ion-forming methemoglobin or nitroso compounds, potential carcinogenicity of nitrosamines thus formed, and the strong bonding of NO and hemoglobin with essentially irreversible formation of nitroxyhemoglobin. He presents a good review of accidental exposures and a thorough clinical review of 11 papers. He distinguishes four types of response: superacute (asphyxia, possibly including methemoglobinemia); acute (the "irritant gas" response); delayed (pulmonary edema); and subacute (bronchitis and bronchiolitis, bronchiolitis obliterans, pneumonia). He notes that acute bronchial spasm, diffuse alveolar damage with pulmonary edema, and bronchiolitis obliterans can occur in sequence or isolation, further confusing the clinical picture.

Of the epidemiologic evidence concerning chronic low-level exposure, he considers the Chattanooga schools study the best, despite its known deficiencies, and accepts its finding of effects at an annual mean of 0.08 ppm NO_2 (Shy et al.,[81-83] Pearlman,[75] Warner and Stevens[96]). He is dubious about the Rosice, Czechoslovakia study of

Petr and Schmidt[76] with its "obscure hematologic indices of very questionable significance" and rejects the early occupational study of Vigdortschik et al.[90] entirely.

Guidotti writes of wide variations in respc e between animal species and within species. The concentration of NO_2 in the lower lung may be substantially less than that inhaled. In discussing low-level effects he quotes, inter alia, Carson et al.[12] and Dowell et al.[22] (q.v. in this report). Many effects are discussed, by target or response: cilia, macrophages, bronchospasm, etc. Potentiation of respiratory infection by NO_2 is well reviewed and he notes that Cooper and Tabershaw[18] considered this might be "the most sensitive indicator so far discovered for a biological effect of NO_2."

Guidotti strongly questions the Air Quality Standard of 0.05 ppm annual average (vs. the Chattanooga response at 0.08 ppm) and, less strongly (because "exposure is brief"), the occupational standard of 5 ppm.

Comment:

This is a competent and up-to-date review, particularly from the medical viewpoint. The most notable deficiency is lack of discussion about peak vs. average exposure and intermittent vs. continuous exposure. His acceptance of the Chattanooga response at 0.08 ppm may be questioned because of known deficiencies in the study.[96]

He refers to wide variations in response between animal species and within species; our own review suggests that the variations are not wide.

• Mueller PK, Hitchcock M: Air quality criteria--toxicological appraisal for oxidants, nitrogen oxides, and hydrocarbons. J Air Pollut Control Assoc 19:670-676, 1969

The authors summarize effects of NO_x as sensory irritation, lung inflammation, oxidation of lung tissue, and ultra-structural changes. Although effects are reversible over days, "The suggestion is very strong that repeated exposure to NO_2 or O_3 can produce a cumulative effect. The finding in animals that low levels of NO_2 initiate inflammation implies an acceleration of the somatic cell turn-over rate which is also a factor in the aging process." The oxidizing process also "creates agents which have the potential for cross-linking structural proteins... (a) factor in aging." Pre-emphysematous lesions have been induced by long exposure to rats.

• National Academy of Sciences, National Research Council, Committee on Toxicology: Guide for Short-Term Exposures of the Public to Air Pollutants. 1, Guide for Oxides of Nitrogen. Prepared under EPA Contract No. CPA 70-57. Washington, D.C., April 1971

The paper quotes Elkins' statement (J Ind Hyg Toxicol 28:37-39, 1946) that NO is nonirritant and Stokinger's statement (in Air Pollution,

A.C. Stern, ed., Vol. I, Academic Press, N.Y., 1962, pp. 282-334) that it is one-fifth as toxic as NO_2. N_2O_5 is briefly mentioned. NO_2 is "the oxide of nitrogen of primary concern." Cofactors in response to NO_2 are temperature (positive effect of hot or cold environment), predisposing chronic destructive pulmonary disease (quoting von Nieding), heredity, and age.

The paper finds a conflict between (a) the work of Ehrlich and of Coffin on increased susceptibility to bacterial infection and (b) Wagner's[95] finding of no difference in exposed vs. control rats from a colony having spontaneous pulmonary infection.

The paper summarizes the effects of NO_2: deep lung irritant (edema, bronchiolitis obliterans), additive with other irritants; lipoperoxidation, reversible alteration in mast cell morphology after a single, brief low-level exposure; ciliastasis; increased response to respiratory infection; and possible acceleration of spontaneous tumor formation.

The paper recommends:

Short-term public limits

10 minutes	1 ppm
30 minutes	1 ppm
60 minutes	1 ppm
5 hours/day, 3-4 days/month	0.5 ppm
1 hours/day/year	1 ppm

Public emergency limits

10 minutes	5 ppm
30 minutes	3 ppm
60 minutes	2 ppm

(Comment: The rationale is supported by comments but is not fully explicit.)

The paper reviews 28 research reports on NO_2 in an Appendix; most are included in the present report. Comments by NAS are of interest. The tolerance to intense challenge induced by previous exposure is, NAS notes, at the cost of increased thickness of the "air-blood barrier" observed by Dillmann et al.;[20] the implication is impaired gas exchange. Half a dozen papers from Freeman's laboratory are reviewed and the NAS comments that the emphasis "is on morphological changes in the lung tissue, and unfortunately almost no biochemical parameters were measured...."

NAS comments in general on animal exposure, noting that discrepancies between experiments may be due to animal variation, the biochemical parameters measured, tolerance, other contaminants, or differences in exposure schedule. Continuous exposure is found more harmful than interrupted exposure, even if intermittent exposure was at higher concentration.

NAS also reviews human exposures, including volunteers at low-level and acute intense accidents.

• Tabershaw IR, Ottobini F, Cooper WC: Oxidants: air quality criteria based on health effects. J Occup Med 10:464-480, 1968

This review is concerned with chronic effects of oxidants generally (it was given at an Air Quality Symposium), but includes some comments on NO_2 that are worth quoting; also, the comments by D.V. Bates and D.L. Coffin at that meeting are of interest.

The authors noted that eye irritation and lacrimation from exposure to photochemical smog (Comment: This includes NO_2, but ozone and peroxyacyl nitrates may be more important) occurred on more than 200 days a year in Los Angeles, but there was almost no evidence of consequent chronic disease. They refer to the phenomenon of cross-protection, whereby a single brief exposure of animals to O_3 confers increased tolerance to NO_2 (as well as to O_2); exposure to NO_2 is also selfprotecting.

The authors comment on early deficiencies in analysis of NO_x and state that "papers published before 1955 should be considered suspect." The effect on the upper respiratory tract is negligible because NO_2, like other gases of low solubility, passes "through the relatively dry trachea and bronchi into the moisture laden alveoli...."

There is a clear description of the pathology of bronchiolitis obliterans, a condition nonspecific for irritants but most characteristic of NO_2. The authors accept animal models as valid for man, despite species differences; they discount the finding by Wagner et al.[95] of lung tumor acceleration, noting that NO_2 "is not a strong free radical producer." They state, "While there is no evidence that NO_2 is a cumulative poison...we should remain aware that chronic effects may occur."

The authors comment on the probable importance of peak vs. long-term average exposure.

Bates (ibid.), commenting on the paper, drew attention to the demonstration of Macklem and Mead[59] that forced expiratory volume and total airway resistance determined by body plethysmography would be very insensitive indicators of a considerable change in diameter of small airways and would thus miss acute or chronic effects "until they were extremely severe."

Coffin (ibid.) stated "the most important aspect of NO_2 toxicity is its apparent cumulative effect," and quoted Ehrlich's work on intermittent and continuous exposure in potentiating respiratory infection, and the work of Freeman on emphysema in rats; levels as low as 0.6 to 2 ppm may be involved. He also quoted Buell et al. on reversible changes in lung elastin and collagen after 1 hour exposure (at 1 ppm; Coffin did not give the concentration).

APPENDIX B

SUMMARY TABLES OF EFFECTS

TABLE B-1. Human Exposure to NO_2

Conc., ppm	Time, min	Effects	Reference
250	4.7	Collagen degradation, methemoglobinemia, tightness in chest, dyspnea, nonproductive cough, retrosternal burning sensation	Hatton et al.[46] (1977)
158	10	Intolerable; coughing, irritation of nasal and laryngeal mucosa; lacrimation; headache; nausea, vomiting. Subsided after 7 hours, no delayed or long-term effect	Lehmann et al.[56] (1913)
70	20	2/2 hospitalized, pulmonary edema; 1 retired on disability	Mangold et al.[60] (1971)
60	60	Laryngeal irritation; increased respiration rate	Lehmann et al.[56] (1913)
25-100	120	Marked mucosal irritation; increased pulse and respiratory rate	Lehmann et al.[56] (1913)
45 (with 90 ppm NO)	30 followed by 15	Subject hospitalized 7 days, pulmonary edema; no clinical sign at 73 days	Norwood et al.[70] (1956)
20 (with 80 ppm NO)			
50	1	Pulmonary discomfort; nasal irritation (more intense than at 25 ppm for 5 min); substernal pain (2/7 subjects)	Meyers et al.[63] (1961)
25	5	Pulmonary discomfort	Meyers et al.[63] (1961)
5	15	Threshold for decreased arterial partial pressure of O_2 and decreased diffusion capacity for CO in bronchitics	von Nieding et al.[93] (1973)
4-5	10	Lung compliance decreased and airway resistance increased in healthy subjects	Abel (1967)
1-6	15	Threshold for increas i airway resistance in bronchitics	von Nieding et al.[93] (1973

TABLE B-2. Experimental Animal Exposure to NO_2 at Lethal and High Sublethal Levels

Species	Concentration, ppm	Duration, min	Ct, ppm min	Deaths, Percent	Reference
Mouse	1,880	5	9,400	50	DiPasquale[21]
	200	5	1,000	67	Hine[50]
	200	10	2,000	100	Hine[50]
	200	20	4,000	100	Hine[50]
	125	5	625	0	Hine[50]
	125	30	3,750	67	Hine[50]
	125	60	7,500	100	Hine[50]
	100	30	3,000	20	Hine[50]
	100	60	6,000	80	Hine[50]
	75	60	4,500	17	Hine[50]
	50	60	3,000	0	Hine[50]
Rat	1,445	2	2,890	50	Gray[40]
	833	5	4,165	50	Gray[40]
	831	5	4,155	50	DiPasquale[21]
	420	15	6,300	50	Gray[40]
	416	5	2,080	50	Carson[12]
	250	5-10	1,250-2,500	50	Hine[50]
	240	20	4,800	100	Hine[50]
	201	15	3,015	50	Carson[12]
	200	5	1,000	50	Hine[50]
	200	10	2,000	75	Hine[50]
	200	20	4,000	100	Hine[50]
	200	30	6,000	100	Hine[50]
	174	30	5,220	50	Gray[40]
	168	60	9,840	50	Gray[40]
	162	30	4,860	50	Carson[12]
	150	30	4,500	20	Hine[50]
	150	60	9,000	77	Hine[50]
	115	60	6,900	50	Carson[12]
	100	60	6,000	60	Hine[50]
	85	60	5,100	50	Hine[50]
	75	60	4,500	10	Hine[50]
	72	60	4,320	0	Carson[12]

TABLE B-2 (continued)

Species	Concentration, ppm	Duration, min	Ct, ppm min	Deaths, Percent	Reference
Guinea Pig	200	5	1,000	100	Hine[50]
	150	5	750	75	Hine[50]
	100	30	3,000	50	Hine[50]
	100	60	6,000	100	Hine[50]
	75	60	4,500	25	Hine[50]
	50	60	3,000	17	Hine[50]
Rabbit	315	15	4,725	50	Carson[12]
	200	5	1,000	0	Hine[50]
	200	10	2,000	50	Hine[50]
	200	20	4,000	50	Hine[50]
	150	5	750	0	Hine[50]
	150	60	9,000	17	Hine[50]
	100	30	3,000	33	Hine[50]
	100	60	6,000	0	Hine[50]
	75	60	4,500	13	Hine[50]
	50	60	3,000	0	Hine[50]
Dog	200	20	4,000	100	Hine[50]
	ca. 170	15	2,550	50	Carson[12]
	150	60	9,000	67	Hine[50]
	125	5	625	0	Carson[12]
	ca. 106	60	6,360	50	Carson[12]
	100	30	3,000	0	Hine[50]

TABLE B-3. Animal Exposure to NO_2 at Sublethal Dosage Levels

Species	Concentration, ppm	Duration, min	Ct, ppm,min	Effect	Reference
Mouse	125	5	625	Eye irritation	Hine[50]
	50	60	3,000	Increased respiratory rate	Hine[50]
Rat	190	5	950	Severe distress, eye irritation, increased lung weight (ca. ½ LC_{50})	Carson[12]
	104	5	520	Some distress, increased lung weight (ca. ½ LC_{50})	
	100	30	3,000	Eye irritation, increased respiration rate	Hine[50]
	90	15	1,350	Severe distress, eye irritation, increased lung weight (ca. ½ LC_{50})	Carson[12]
	74	5	370	No toxic effect	Carson[12]
	72	60	4,320	Severe distress, eye irritation, increased lung weight (ca. ½ LC_{50})	Carson[12]
	65	15	975	Some distress, increased lung weight (ca. ½ LC_{50})	Carson[12]
	65	120	7,800		Hine[50]
	50	60	3,000	Eye irritation	Hine[50]
	50	120	6,000		Hine[50]
	33	15	495	No toxic effect	Carson[12]
	28	60	1,680	Mild nasal irritation (ca. ½ LC_{50})	Carson[12]
Rabbit	200	5	1,000	Eye irritation, increased respiration rate	Hine[50]
	150	5	750	"	"
	50	60	3,000	"	"
Guinea Pig	40	60	2,400		Hine[50]
Dog	164	5	820	Mild sensory effect (5-min LC_{50} was ca. 328 ppm)	Carson[12]
	125	5	625	"	"
	100	30	3,000	Eye irritation	Hine[50]
	85	15	1,275	Some distress, mild cough, eye irritation	Carson[12]
	75	60	4,500	Eye irritation	Hine[50]
	75	120	9,000	Eye irritation	Hine[50]
	52	15	780	Mild sensory effect	Hine[50]

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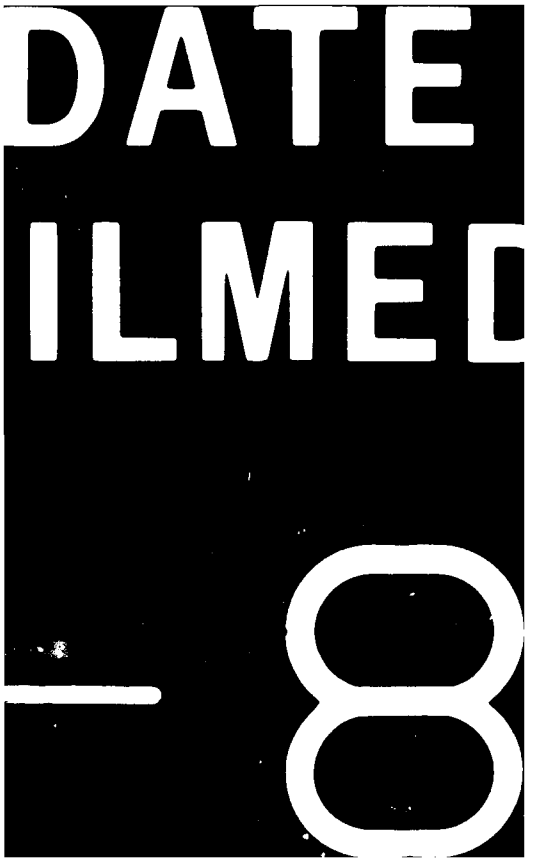
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